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A molecular genome scan and positional candidate gene analysis for important economic traits in the pig

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

Pork quality comprises a set of key fresh meat quality, processing and sensory characteristics that are important for the future profitability and competitiveness of the swine industry. In the past, leanness was considered one of the most important traits (Sellier and Rothschild, 1991). As a result, dramatic improvements in the body composition of pigs have been made. Pork is now leaner than ever, with lower average fat content. Now that the major objective of reducing back fat has been largely achieved, it has been shown that lean meat is not always associated with good meat quality (Cameron, 1990; Hovenier et al., 1992). In order to shift breeding programs towards improvement of meat quality, several other traits must be considered. These traits include intramuscular fat, cholesterol content, ultimate pH, color, water holding capacity or drip loss, tenderness, cooking loss, and sensory traits involving taste (Sellier, 1998).

Conventional selection methods using phenotypic information to assist in the selection of animals with favorable alleles, have been employed for a long time to improve traits in farm animals. However, genetic improvement of meat quality is difficult by traditional breeding programs because of the measurement difficulties in live animals, cost of trait measurements, and a low heritability for some meat quality traits. One strategy to overcome this limitation is to find the specific genes responsible or associated with such traits, to develop genetic markers or genetic tests for these genes, and to use them to select animals early in life.

Detailed knowledge of molecular biology and genomics has facilitated our understanding of different ways to dissect the genetics of complex traits. The development and application of molecular biology techniques to farm animals have progressed rapidly over the past decade. Genes and QTL (quantitative trait loci) affecting performance traits have been found and genetic maps have been expanded greatly. At present, approximately 4,000 loci have been mapped in the pig, with a majority of these being anonymous molecular markers (http://www.ri.bbsrc.ac.uk/pigmap/).

Molecular genetic markers can be used for QTL analysis with individual chromosome scans or genome scans on F2 populations from crosses between divergent breeds. A three generation resource family of a cross between the Berkshire and Yorkshire breed was used for the present study (Malek et al., 2001). This cross allowed us to identify chromosomal regions and genes responsible for differences in meat quality traits between these two breeds.

Although genome scans can identify chromosomal regions that contain QTL that control meat quality and muscle composition traits, this approach has limited map resolution. Therefore, following a genome scan a candidate gene approach can be used to fine map potential QTL.

Known functional roles of genes and genomic locations in species that are rich in genome information, such as human, can be used to find positional candidate genes (Lander et al., 2001). Opportunities for comparative mapping have grown rapidly over the past few years. Currently, a need exists to improve the gene map of the pig. This can be done by mapping specific genes to chromosomal regions in the human and the pig using bi-directional chromosomal painting (Goureau et al., 1996). Comparative mapping will then allow researchers to take advantage of the significant resources in the human genome project.

The research reported in this thesis has two main objectives. The first is to identify chromosomal regions of the pig associated with growth, meat quality and sensory traits based on a molecular genome scan analysis of a three generation Berkshire x Yorkshire cross. The second is a candidate gene analysis to find genes that are associated with traits of interest.

Thesis Organization

This thesis is written in the alternative format in which a general introduction and a general literature review (Chapter 1) are followed by five manuscripts for publication in scientific journals (Chapters 2, 3, 4, 5 and 6). The final chapter, Chapter 7, gives conclusions, recommendations, and implications for future work. Appendix A includes a manuscript that was prepared by Dr. Elisabeth Huff-Lonergan as first author (and Malek as co-author) on phenotypic analysis of the meat quality traits in the F2 population that was the basis of this thesis. Relevant references are compiled for each chapter, followed by the tables and figures. Papers were prepared for publication in Mammalian Genome (Chapters 2, 3, 5 and 6) and the Journal of Animal Science (Chapter 4). Therefore, the organization of the papers is in accordance with the format of those journals.

Literature Review

Pork Quality

Pork is one of the important protein sources worldwide. The pork industry has been progressing rapidly to meet the demand for improved meat quality, which is one of today's key consumer concerns. Most traits of economic importance, including meat quality, are

quantitative traits that are likely controlled by a fairly large number of genes. However, some of these genes might have a large effect. Such genes are called major genes.

Meat quality is an important profit factor for producers. Producers that have pigs with better pork quality, will find a higher market price. Good meat quality is generally defined as darker color, more water holding capacity, and better texture, which describes meat that is free of the PSE syndrome (pale, soft, and exudative) (Penny 1969; Wariss and Brown 1987). However, meat quality cannot be defined using only one parameter such as appearance. The following are the most important factors considered in this study that have been evaluated in order to assess growth, body composition and meat quality:

1. Body composition: measurement of lean and fat content (tenth rib back fat, last rib back fat, lumbar back fat, loin eye area, total lipid, and cholesterol).
2. Performance: birth weight, 16 day weight, average daily gain, feed efficiency.
3. Subjective carcass evaluation: loin muscle marbling, color and firmness of the meat following slaughter.
4. Light reflectance: measurement of the light reflectance off the surface of the muscle such as Minolta reflectance and Hunter L.
5. Drip loss or water holding capacity: measurement of the ability of muscle to hold water.
6. Tissue quality: measurement of different muscle fiber types (ratio of type IIa/IIb myosin heavy chains).
7. Cooking and sensory panel evaluation: objective evaluation of meat quality for firmness and texture (tenderness, chewiness, and juiciness), taste profile (sweet, sour, salty, and bitter), and flavor (roasted, nutty, and meaty).

8. Glycolytic potential: estimation of muscle glycogen and lactate content at slaughter.
9. Muscle pH: measurement of pH at 24 and 48 hours after slaughter.
10. Tenderness measures: Star Probe Force method, chew and tenderness scores.

For the research described in this thesis, body composition and meat quality were evaluated on the basis of 40 traits (Malek et al., 2001), in order to locate QTL affecting these traits in the pig genome.

Gene mapping

One of the main ideas behind genomic research in farm animals has been the mapping and identification of genes underlying economically important traits (Andersson, 2001). The localization of genes and QTL to chromosomal regions flanked by markers may facilitate the application of marker assisted selection to improve body composition and meat quality. The use of these markers also increases the accuracy of selection, especially for traits with low heritability (Falconer and Mackay, 1996).

In order to map these markers, individuals from the population under study must be genotyped. Genetic markers are identifiable DNA sequences that facilitate the study of inheritance of a trait or a gene. It is likely that the majority of anonymous genetic markers will have no effect on performance traits themselves. But such markers allow us to identify areas of the genome containing important genes. Genes closely linked to the marker will generally be inherited with it. Genetic markers will help us trace regions of chromosomes from parents to offspring. Therefore, genotyping animals for genetic markers is an investment with the final aim to better assess true genetic merit of animals. An immediate question to be faced is how to choose an appropriate genetic marker for a specific investigation. Generally speaking, the following factors (Dietrich et al., 1992) must be

considered in the use of markers in their practical application: 1) development and genotyping costs, 2) degree of polymorphisms, 3) reliability and reproducibility, 4) ability to automate genotyping , and 5) the number of samples that can be run per day. A variety of genetic markers are currently in use, including restriction fragment length polymorphisms (RFLPs) (Gordzicker, 1974; Paterson et al., 1988), multiple arbitrary amplicon profiling (MAAP) (Gustavo and Peter, 1998), randomly amplified polymorphism DNA (RAPDs) (Williams et al., 1990), variable number of tandem repeats (VNTRs) (Nakmura , 1987), amplified fragment length polymorphisms (AFLPs)(Meksem et al., 1995; Thomas et al., 1995), single strand conformational polymorphisms (SSCPs)(Ainsworth, 1991), minisatellites, and microsatellites (McAlpine et al., 1989). Microsatellite markers are common for genome mapping because they are highly polymorphic, evenly dispersed in the genome, and detectable by PCR based assay (Dear, 1997; Bishop et al., 1995).

The genome scan and candidate gene approach are two approaches that allow us to identify the genes that influence traits of interest in the pig (Rothschild and Plastow, 1999).

Genome scan approach

The genome scan approach uses anonymous genetic markers spread over the genome. Genome scans can be employed to identify chromosomal regions that control quantitative traits of economic importance. The choice of experimental designs for the detection and mapping of QTL depends on the following factors: 1) species, 2) availability of a resource family with a large number of animals, and 3) availability of informative genetic markers, evenly spaced to cover the entire genome.

Comprehensive genetic linkage maps for the pig have been developed and expanded greatly over the past years with an international mapping effort (Archibald et al., 1994;

Archibald, personal communication) together with a USDA/ARS effort (Rohrer et al., 1996). The genome coverage of these genetic maps for genes and markers is sufficient to allow researchers to conduct QTL linkage analysis.

Resource Family. In order to investigate the possibility of finding swine QTL, a resource family is required and genetic markers must be genotyped for this family. Better (more polymorphic) markers and larger resource families will aid in dissecting traits. A limited number of studies have attempted to map QTL for meat quality traits but they have generally involved a cross of a Western breed to either Chinese breeds (e.g. Meishan), or the Wild Boar (Andersson-Eklund et al., 1998; Milan et al., 1998; Moser et al., 1998; Wang et al., 1998; Yu et al., 1999; De Koning et al., 2000a, b). These studies have reported the existence of QTL for meat quality traits on almost all chromosomes, except 10, 17, and 18. The QTL detected in such crosses may, however, not be of immediate practical interest because of the poor performance of the exotic breeds involved in these crosses for several traits of importance to modern swine breeding.

The Berkshire and Yorkshire are breeds of real commercial interest that have demonstrated considerable differences in meat quality, with Berkshire pigs having very good meat quality traits (Goodwin and Burroughs, 1995). In order to identify the chromosomal regions and genes responsible for differences in meat quality traits in these breeds, a three generation resource family was developed (Malek et al., 2001).

Statistical Analysis for QTL detection. Many statistical methods have been developed for the detection and mapping of QTL: Maximum Likelihood (ML) (Bovenhuis and Weller, 1994; Mackinnon and Weller, 1995), BLUP based methods like REML (Fernando and Grossman, 1989), Bayesian analysis using Gibbs sampling approaches

(Metropolis et al., 1953), and least squares (LS) methods, (Haley and Knott, 1992). The ML (Lander and Botstein, 1989) and LS (Haley and Knott, 1992) methods are the two most common methods used for interval mapping based on genetic markers. Although the ML method is one of the best methods, it is computationally intensive and cannot be applied to complicated situations. The LS method involves simple regression of phenotype on genotype and is easy to use for detecting the presence of a QTL linked to a marker (Whittaker et al., 1996).

Because of the large number of correlated statistical tests conducted and associated concerns about false positive claims for QTL when using interval mapping, relevant significant thresholds must be calculated. Churchill and Doerge (1994) developed a permutation test to derive significance thresholds of the F statistic at the chromosome and genome-wise levels for a single trait. Computing significance threshold for all traits requires too many computations. Therefore, a permutation test can be done for selected traits (Lee et al., 2001).

Candidate gene approach

The second approach to finding genes is the candidate gene approach (Rothschild and Soller, 1997). The hypothesis used in this approach assumes that candidate genes represent a large proportion of the QTL that determine a particular trait. Candidate gene analyses are usually single generation studies and they can be applied to any random mating population that can be phenotyped. Therefore, this approach is an ideal approach for QTL identification in commercial breeds.

The following are steps are involved in a candidate gene analysis (Rothschild and Soller 1997): 1) selection of a candidate gene, 2) database analysis of the known genomic

organization of the candidate gene in another species, 3) designing primers from the known sequence, 4) sequencing of the PCR product for gene verification, 5) amplifying pooled genomic DNA samples to check for polymorphisms, 6) designing a PCR-RFLP test to allow amplification and analysis of large numbers of individuals, and 7) analysis of associations between traits of interest and genotype for the candidate gene.

To identify a candidate gene (step one from above), two sources of information can be used (Rothschild and Soller, 1997):

Physiological approach. This is an experimental approach in which prior knowledge of the biochemistry and/or physiology of a trait is used to draw up a list of genes whose products could affect the trait of interest.

Positional approach. The positional candidate gene approach is currently is a successful methods of identifying a gene for a trait of interest. This approach allows researchers to combine information about a gene's chromosomal location for easier identification of a potential causative gene (Rothschild et al., 1997). The positional candidate gene approach relies on a three step process: 1) localizing the area of interest to a chromosomal sub region, generally based on a genome scan analysis, 2) searching databases for an attractive candidate gene within that sub region, based on comparative mapping, 3) testing the candidate gene for causative mutations.

Comparative mapping. Comparative mapping, using genomic locations of known genes in another species to map a gene in the species of interest (step 2), has grown rapidly over the past few years. Comparative mapping is one of the approaches to fine map a chromosomal region and improve our ability to find genes responsible for traits. Currently, a need exists to improve the pig gene map by mapping specific genes that have been mapped

onto the human genome. This will allow swine geneticists to take advantage of the significant resources in the human genome project.

In the last step, genotypes and phenotypic trait measurements are used for statistical analysis to look for possible associations between a particular phenotype and the genotype of the candidate gene. This can be done by testing for association(s) between existing polymorphisms (different alleles) and the trait of interest (Neimann-Sorensen and Robertson, 1961; Ron et al., 1994) using mixed linear model procedures with the alleles or genotypes as fixed effect SAS (SAS/STAT, 1990). The gene effect detected by this approach must be confirmed in other populations, in particular those involving breeds that are of commercial (economic) interest.

Due to the rapid development of molecular biology over the past two decades, several genes have been found affecting meat quality. The Halothane (HAL) or stress gene is responsible for porcine stress syndrome in pigs, which has with major effects on meat quality (Christian, 1972; Eikelenboom and Minkema, 1974). Fujii et al. (1991) discovered the causative mutation responsible for this phenotype due to abnormalities in the Ca2+ release channel of skeletal muscle sarcoplasmic reticulum (the ryanodine receptor). This was the first application of a DNA test for a major gene in pig breeding. The Rendement Napole (RN) or acid meat gene, is another important major gene affecting meat quality, (Monin and Sellier, 1985; LeRoy et al., 1990; Milan et al., 2000). This (R200Q) substitution in the RN *(PRKAG3)* gene caused a 70% increase in glycogen in muscle in RN^- homozygous and heterozygous animals that then resulted in the observed lower muscle pH 24 hrs after slaughter, reduced water holding capacity in the muscle and much lower yield of a cured cooked ham product. The 200Q allele is associated with all RN^- animals and was present

primarily in the Hampshire pig breed but not in pigs with an rn^+ phenotype or in other breeds (Milan et al., 2000). Other genes investigated for meat quality have included H-FABP, and A-FABP, which may be associated with intramuscular fat (Gerbens et al., 1997).

Candidate genes for meat quality on pig chromosome 5

The detection of several QTL for meat quality and body composition on chromosome 5 in the pig (Malek et al., 2001) made this chromosome attractive for a positional candidate gene approach. This approach will help to increase the number of genes mapped in this region, allow a fine map to be developed, and make it possible to investigate possible candidate genes.

We investigated five genes that were selected based on function and their predicted location on pig chromosome 5 (SSC5) or SSC14 based human comparative mapping using bi-directional chromosome painting work (Goureau et al., 1996). These genes were the: 1) the protein phosphatase (***PPP1CC***) gene, 2) the G protein-coupled receptor 49, glycoprotein hormone receptors subfamily (***GPR49***) gene, 3) the Acetyl-CoA carboxylase beta promoter (***ACACB***) gene, 4) the Dual specificity phosphatase 6 (***DUSP6***) gene, and 5) the ATPase, Ca^{2+} transporting, plasma membrane 1 (***ATP2B1***) gene. These genes are described in further detail below.

Protein phosphatase-1, catalytic subunit, gamma isoform gene (*PPP1CC*)

Type-1 protein phosphatase is essential for cell division and it participates in the regulation of glycogen metabolism, muscle contractility, and protein synthesis (Villa Moruzzi et al., 1996; Raghavan et al., 2000). This gene comprises a catalytic subunit, pp1-alpha, -beta or –gamma (Okano et al., 1997). *PPP1CC* activity is reduced in skeletal muscle from human subjects with insulin resistance (Norman and Mott, 1994).

G protein-coupled receptor 49, glycoprotein hormone receptor subfamily gene *(GPR49)*

This is a novel member of the glycoprotein hormone receptor subfamily. The *GPR49* gene with the glycoprotein hormone receptors contains a long extracellular domain with a total of 16 leucine-rich repeats (Hong et al., 1999). Northern blot analysis showed that *GPR49* was expressed in skeletal muscle, placenta, spinal cord, and various regions of the brain (McDonald, 1998). The *GPR49* produced protein is most likely a receptor for a novel class of glycoprotein ligands.

Acetyl-CoA carboxylase gene *(ACACB)*

Acetyl-CoA carboxylase (ACC), an important enzyme in fatty acid biosynthesis, is present in at least two isoforms (Abu-Elheiga et al., 2000). The beta-isoform of acetyl-Coenzyme A carboxylase (ACACB) is predominantly expressed in heart and skeletal muscle, whereas the alpha-isoform (ACACA) is the major ACC in lipogenic tissues (Lee et al., 2001). The *ACACB* gene may be involved in the provision of malonyl-CoA or in the regulation of fatty acid oxidation (Saddik, 1993). This gene is involved in the first step (pathway) in long-chain fatty acid synthesis.

Dual specificity phosphatase 6 gene (*DUSP6*)

The protein encoded by *DUSP6* is a member of the dual specificity protein phosphatase subfamily (Smith et al., 1997). These phosphatases inactivate their target kinases by dephosphorylating both the phosphoserine/threonine and phosphotyrosine residues (Smith et al., 1997). They negatively regulate members of the mitogen-activated protein (MAP) kinase superfamily, which are associated with cellular proliferation and differentiation. Different members of the family of dual specificity phosphatases show distinct substrate

specificities for various MAP kinases, different tissue distribution and subcellular localization, and different modes of inducibility of their expression by extracellular stimuli (Toyota et al., 2000).

ATPase, Ca^{2+} transporting, plasma membrane 1 gene (*ATP2B1*)

Human plasma membrane Ca^{2+} -ATPase (*ATP2B1*) is expressed in neuronal tissue and skeletal muscle and plays an important role in fine-tuning of the intracellular concentration of Ca^{2+} (Gromadzinska et al., 2001). This enzyme exhibits a high degree of tissue specificity and is regulated by several mechanisms. Protein phosphatases decrease both the basal activity of Ca^{2+}-ATPase and its affinity for calmodulin. *ATP2B1* isoforms are encoded for by at least four separate genes and the diversity of these enzymes is further increased by alternative splicing of transcripts (Olson, 1991).

Summary

The research effort described in this Ph.D. thesis will help increase the body of knowledge on genes that control meat quality. There is no question that several genetic markers affecting meat quality will be used in the future. The use of these genetic markers will help to increase the speed and efficiency of genetic improvement in a population. The consumer will be the ultimate beneficiary in terms of improved pork quality.

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CHAPTER 2. A MOLECULAR GENOME SCAN ANALYSIS TO IDENTIFY CHROMOSOMAL REGIONS INFLUENCING ECONOMIC TRAITS IN THE PIG. I. GROWTH AND BODY COMPOSITION

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Abstract

Genome scans can be employed to identify chromosomal regions and eventually genes (quantitative trait loci or QTL) that control quantitative traits of economic importance. A three-generation resource family was developed by using two Berkshire grand sires and nine Yorkshire grand dams to detect QTL for growth and body composition traits in pigs. A total of 525 F_2 progeny were produced from 65 matings. All F_2 animals were phenotyped for birth weight, 16 day weight, growth rate, carcass weight, carcass length, back fat thickness, and loin eye area. Animals were genotyped for 125 microsatellite markers covering the genome. Least squares regression interval mapping was used for QTL detection. All carcass traits were adjusted for live weight at slaughter. A total of 16 significant QTL, as determined by a permutation test, were detected at the 5% chromosome-wise level for growth traits on Chromosomes (Chrs) 1, 2, 3, 4, 6, 7, 8, 9, 11, 13, 14, and X, of which 2 were significant at the 5% genome-wise level and 2 at the 1% genome-wise level (on Chrs 1, 2, and 4). For composition traits, 20 QTL were significant at the 5% chromosome-wise level (on Chrs 1, 4, 5, 6, 7, 12, 13, 14, 18), of which 1 was significant at the 5% genome-wise level and 3 were

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significant at the 1% genome-wise level (on Chrs 1, 5, and 7). For several QTL the favorable allele originated from the breed with the lower trait mean.

Introduction

The development of molecular biology techniques and the application of these techniques to farm animals have progressed rapidly and have opened new vistas for investigators wishing to identify genes that control quantitative traits (quantitative trait loci or QTL). Comprehensive genetic linkage maps for the pig have been developed over the past few years with an international mapping effort (Archibald et al. 1994; Archibald, personal communication), and a USDA/ARS effort (Rohrer et al. 1996). At present, approximately 2000 genes and markers have been mapped in the pig, with a majority of these being anonymous molecular markers (http://www.ri.bbsrc.ac.uk/pigmap/).

On the basis of these linkage maps and data from F_2 resource families, several recent studies have reported the discovery of a number of QTL affecting growth and body composition traits in the pig on a variety of chromosomes (Andersson et al. 1994; Wang et al. 1998; Rohrer and Keele 1998a, 1998b; Marklund et al. 1999; Paszek et al. 1999; De Koning et al. 1999; Perez-Enciso et al. 2000). Walling et al. (2000) conducted the first joint QTL analysis for growth and back fat on SSC 4 based on a large data set from several F2 crosses and detected several QTL. Recently, QTL analyses have expanded from the search for Mendelian QTL to QTL with non-Mendelian inheritance. This has resulted in the identification of several QTL with imprinted effects for body composition traits (Knott et al. 1998; Jeon et al. 1999; Nezer et al. 1999; De Koning et al. 2000b).

Most QTL studies in pigs to date have involved exotic crosses with breeds of commercial interest crossed either to Chinese breeds (e.g. Meishan), or the Wild Boar. The QTL detected in such crosses are not of immediate practical interest because of the poor performance of the exotic breeds for several traits of importance to modern swine breeding. Resource families using commercial breeds or lines did not exist at the initiation of this project. Therefore, the objectives of this study were to develop a three generation resource family using the Berkshire and Yorkshire breeds and to identify chromosomal regions responsible for breed differences for a comprehensive set of growth, body composition, muscle and meat quality, and sensory traits. Choice of these two commercial breeds was based on results from the National Pork Producers Council Genetic Evaluation Program (Goodwin and Burroughs 1995), which revealed that considerable differences in meat quality exist between commercial breeds and that the Berkshire breed, in particular, has very positive meat quality characteristics. In this paper we present results of the QTL analyses for growth and body composition traits. Results for meat quality traits are presented in a companion paper (Malek et al. 2001). Only single QTL models with Mendelian inheritance were investigated here. Additional statistical analysis to consider multiple QTL, within-breed QTL effects, and gametic imprinting are in progress.

Materials and Methods

Family structure.

A three-generation resource family was created by using two purebred Berkshire grand sires and nine Yorkshire grand dams. The two boars used were from the Casino and Count sire families that are well known within the breed. Sows were mated by artificial insemination at

the Iowa State University Swine Breeding Farm, using semen from two boar studs, to produce nine litters of F_1 individuals. From the 9 F_1 litters, 8 boars and 26 females were chosen to produce the 525 F_2 animals that were used in this study. In total, 65 matings were made to produce four sets of F_2 offspring.

Management.

The F_1 animals that were kept for breeding were put in outside lots with shelter. The F_1 gilts were bred at 8 - 9 months of age, and sows were bred after weaning their respective litters during the course of the experiment. The females farrowed in rooms that contained 12 farrowing crates and were fed a 15% protein lactation diet *ad libitum*. The F_2 pigs were weaned at 16 - 21 days of age. Feed was made available at 10 - 14 days of age. Litters were kept together during the growing and finishing phases. At weaning, males were castrated and the pigs were moved to a nursery, where they received a 21% protein complete feed for 5 - 7 days and then a 20% protein complete feed for 3 weeks. This was changed to an 18% protein ration for another 2 - 3 weeks. When the pigs left the nursery, they were placed in pens that allowed for an average of eight sq. ft. per pig. The diet was changed to an 18.8% protein diet until the pig's weight reached 34 kg on a pen average. At that time, the diet was changed to a 17.5% protein diet until pigs reached 72 kg, and then to a 16% protein diet until the pigs went to market. All diets were fortified with vitamins and minerals for the age of the pig. Water was provided *ad libitum*. Pigs were sent to slaughter at a target weight of 115 kg. The slaughter point was determined by weighing pigs at weekly intervals when they approached 115 kg.

Traits measured.

The traits measured on the live animal included birth weight, 16 day weight, average daily gain from birth to weaning, and average daily gain on test from weaning to slaughter. After slaughter and chilling, carcass traits were evaluated at the plant by trained personnel according to National Pork Producers Council guidelines (NPPC, 1991). Traits recorded for the purpose of the present paper were live weight at slaughter, carcass weight, carcass length, tenth rib back fat, lumbar back fat, last rib back fat, average back fat, and loin eye area. See Table 1 for a description of the traits.

DNA isolation, marker selection, and genotyping.

Blood samples were collected from all F2 animals and their parents (F_1) and grandparents (F_0), and DNA was isolated. Likely parentage (or collection) problems existed for 13 F_2 animals, and these were removed, leaving 512 animals for QTL analysis. Genotyping was subcontracted to a commercial laboratory (GeneSeek Inc, Lincoln, Neb., USA). In total, 180 markers were tested on the F_0 and F_1 animals to determine the final 125 informative markers used for genotyping the F_2 animals (see Table 2).

Marker alleles were amplified by PCR and scored following electrophoresis, using infrared fluorescent technology. Markers were amplified using either end-labeled forward primers or M13-tailed forward primers. Labeled forward primers were synthesized by LI-COR (Lincoln, Neb., USA), while M13-tailed forward primers and all reverse primers were synthesized by Research Genetics (Huntsville, Ala., USA). End-labeled reactions used 25 ng genomic DNA, 200μM of each dNTP, 0.15 picomol of labeled forward primer (either IR700 or IR800; LI-COR, Lincoln, NE), 1 picomol of unlabeled reverse primer, 0.5 U Taq-Gold

polymerase with supplied $MgCl_2$-free buffer (Perkin-Elmer, Foster City, Calif., USA), and 2.5 mM $MgCl_2$. M13-tailed reactions were the same, except that 0.3 picomol of each primer was used. Each forward primer had a 19-bp 5' tail consisting of M13 sequence, and each PCR included 0.3 picomol of a fluorescently labeled 19-bp M13 primer (either IR700 or IR800). The PCR began with a 95°C incubation temperature for 5 min, followed by "touchdown" PCR with annealing temperatures beginning at 68°C and decreasing by 2°C per cycle to 54°C. In total, 33 cycles were performed at a 54°C annealing temperature. PCR ended with a 7min extension at 72°C. PCR products were denatured at 95°C prior to electrophoresis (1500 V, 50mA, 50W, 45°C) in 7.0% denaturing polyacrylamide gels in LI-COR (Model 4200 IR^2) sequencers. Alleles were scored based on size relative to known DNA size standards.

Statistical analysis.

Marker linkage maps were computed with using Crimap version 2.4 software (Green et al. 1990), using the flips and all options to get the best order of the markers and the fixed option to obtain the map distances. The maps were then used for QTL analysis of the 18 autosomes and the X Chr. by using the line cross least squares regression interval mapping program developed by Haley et al. (1994). Marker information was used to calculate the probabilities that an F_2 offspring inherited none, one, or two alleles from each breed for a putative QTL at each 1cM position in the genome. Based on these probabilities, additive and dominance coefficients were derived for the putative QTL, contrasting average QTL alleles from the two breed origins, as represented by the F_0 grandparents. Information content of each marker was

calculated on an individual marker basis and on a linked marker basis. Designating P_{BB} and P_{YY} as the average probability of a given F_2 progeny to have received both marker alleles from the Berkshire and Yorkshire breeds, respectively, polymorphism information content for a given marker was computed as $(P_{BB} - P_{YY})$. Information content on a linked marker basis includes information from flanking markers, in addition to information from the marker itself, for determining the breed origin of marker alleles in F_2 progeny, following Haley et al. (1994).

The least squares regression model used for QTL analysis included the fixed effects of sex and year-season for all traits, along with additive and dominance coefficients for the putative QTL. Litter size was added as a covariable for birth weight, 16 day weight, and for average daily gain from birth to weaning, and live weight was added as a covariable for carcass traits. Note that adjusting carcass weight for live weight corresponds to analysis of carcass yield.

Detection of QTL was based on an F statistic that was computed from sums of squares explained by the additive and dominance coefficients for the QTL. Significance thresholds of the F statistic were derived at the chromosome and genome-wise levels on a single trait basis by the permutation test of Churchill and Doerge (1994). A total of 10,000 random permutations of the data were used. Because computational requirements prevented permutation tests to be conducted for all traits, significance thresholds were derived based on five representative traits: carcass weight, last rib back fat, loin eye area, cholesterol content, and marbling. See Malek et al. (2001) for a description of the latter two traits. Average thresholds across these five traits were used for significance testing for all traits. See Lee et al. (2001) for more details on the permutation tests conducted for this project.

Results and Discussion

Arithmetic means and standard deviations of traits measured on the F_2 animals are listed in Table 1. Trait measurements were within the usual range of scores. Relationships between traits are discussed in Huff-Lonergan et al. (2001, Appendix A).

Chromosome linkage map results.

Marker mapping results are presented by chromosome in Table 2. The 125 markers genotyped in this study represent reasonable genome coverage. The total map length was 20.8 Morgans, which compares well to previous swine linkage maps. In all cases but one, map order of the markers was the same as in the USDA map (Rohrer et al. 1996). The exception was a switch in order for SSC 2 between SW2157 and SW1408. In our map these markers are 4 cM apart, while the order is reversed in the USDA map and they are 2 cM apart. Our results and those of Rohrer et al. (1996), however, differed from those of Paszek et al. (1999) for Chrs 1, 4, 8, and 10. Map lengths for these chromosomes were considerably longer in the study of Paszek et al. (1999). This may have been caused by genotyping errors, which are known to increase map lengths. The average distance in our study between adjacent markers was 17 cM, but 8 gaps existed of greater than 30 cM. Finding markers for these gaps was limited by the need to use markers that were easy to use and informative. Average information content was 0.76 and 0.82 on the individual and linked marker basis (Table 2). For some markers, however, information content on an individual basis was less than 0.5. The lowest information content on a linked marker basis, however, was 0.64.

Significance thresholds.

Individual chromosome significance levels at the 5% level, as determined by the permutation test, differed slightly by trait (Lee et al. 2001), but more substantially by chromosome. For significance testing, average thresholds across the five evaluated traits were used. See the footnote on Table 3 for a list of average thresholds by chromosome. Average 5% chromosome-wise thresholds ranged from 4.34 to 5.32. Thresholds for chromosome-wise significance at the 5% level correspond approximately to suggestive significance at the genome-wise level (De Koning et al. 1999; Lander and Kruglyak 1995).

Genome-wise significance thresholds also differed slightly by trait (Lee et al. 2001). Average genome-wise thresholds across traits were 8.22 and 9.96 for the 5% and 1% levels. Genome-wise threshold values were similar to those obtained by De Koning et al. (1999), who analyzed data with a similar marker density and family structure.

General QTL mapping results.

Estimates for QTL significant at the 5% chromosome-wise level are presented in Tables 3 and 4. The QTL graphs, representing plots of the F statistic across chromosomes, are shown in Fig. 1 for chromosomes with QTL significant at the 5% genome-wise level. Although some graphs suggest evidence for multiple QTL in adjacent intervals for the same trait (Fig. 1E), only results for the most significant position were included in Tables 3 and 4 because only single QTL models were tested.

A total of 36 QTL were detected at the 5% chromosome level for the 11 traits evaluated in this study, not counting potential multiple QTL in adjacent intervals. Over the 11 traits examined we would expect 11 QTL to be significant at the 5% chromosome-wise

level by chance alone. Thus, over three times as many QTL were detected at this level than wereexpected by chance.

Of the 36 suggestive QTL, 3 and 5 QTL were significant at the 5% and 1% genome-wise levels (Table 4). Over the 11 traits examined, we would expect 0.5 and 0.1 QTL to be significant at these levels by chance alone. Thus, clearly, more QTL were identified at these levels than were expected. In addition, several of the QTL found here have been identified in previous studies based on exotic crosses, as will be discussed in the following on a trait by trait basis. Other QTL found in this study have not been identified previously and vice versa. Differences between this and previous studies may be the result of false negatives and false positives in this or literature studies, or may be due to differences in QTL that segregate between the different breeds used.

There were QTL identified at the 5% chromosome level for nearly all traits and on all chromosomes except 10, 12, 15, 16, and 17 (Tables 3 and 4). Most QTL accounted for 3 - 5% of the F_2 variance, but some reached 7% (Table 3). Note that these variance estimates may be biased upward because they are based on only significant results. Total trait variances explained by QTL reported in Table 4 may, however, be biased downward because potential multiple QTL in adjacent regions were ignored.

A priori we might expect to find fewer QTL in this cross of commercial breeds compared with the divergent crosses reported on in previous studies, which involved an exotic breed (Wild Boar or Chinese breeds). Expected differences between the Berkshire and Yorkshire breeds for the traits evaluated here are given in Table 1, based on crossbred results from the National Pork Producers Council genetic evaluation program (Goodwin and

Burroughs 1995). It is recognized, though, that the grandparents used in our cross represent only a small sample of their respective breeds.

Birth weight.

Only one suggestive QTL at the genome-wise level, which is equivalent to significance at the 5% chromosome-wise level, was detected for birth weight. This QTL was on SSC 3 (Tables 3 and 4). The additive effect suggested that Berkshire alleles tended to be associated with lower birth weight in comparison with Yorkshires, but heterozygotes had the lowest birth weight (Table 3). The variance accounted for by this QTL was 2.9%. There are no previous reports of QTL affecting birth weight on SSC 3. However, Paszek et al. (1999) found suggestive QTL for birth weight on SSC 4, 5, 6, 9, and 16. Also, Rothschild et al. (1995) found an association of the TNFalpha gene with birth weight. This gene lies within the swine major histocompatibility complex on SSC 7.

Average daily gain.

In total, five QTL were detected for average daily gain to weaning and average daily gain on test, of which two were significant at the 5% genome-wise level (Table 4, Fig. 1B, 1C). Berkshire alleles were superior to Yorkshire alleles for three of five QTL (Table 3). Heterozygotes had greatest growth for two out of five QTL. One QTL was for average daily gain to weaning, on SSC 9. The other four QTL were for average daily gain from weaning to slaughter, on SSC 2, SSC 4, SSC 8, and SSC 9.

The QTL on SSC 4 confirms results of several other studies that found a QTL for late growth in a similar region of SSC 4 (Andersson et al. 1994; Knott et al. 1998; Milan et al.

1998; Wang et al. 1998; Marklund et al. 1999; Paszek et al. 1999; Walling et al. 2000). Rohrer (2000) did not find evidence of a QTL for growth on SSC 4. Paszek et al. (1999) reported 30 QTL for various early and late growth traits on SSC 1, 5, 6, 7, 10, 11, 12, 13, and 16. None of these were confirmed in this study. Their QTL on SSC 13 was also found by Andersson et al. (1994), Knott et al. (1998), and Yu et al. (1999). Paszek et al. (1999) also found 11 QTL for late growth, on SSC 2, 4, and 8. We were able to confirm some of these, with QTL detected in similar regions on SSC 2, 4, and 8. Rohrer (2000) reported a QTL on SSC 1 (at 128 -134 cM) that significantly affected early growth, which confirmed the result of Paszek et al. (2000). Milan et al. (1998), Wang et al. (1998), and Rohrer (2000) also reported a QTL for late growth on SSC 7 that was not confirmed in our study. Cassas-Carrillo et al. (1997) reported the detection of QTL affecting late growth on SSC 3. We did not find the same result, but their QTL for growth and the QTL for birth weight from our study (Table 3) were mapped to the same region. We also detected two suggestive QTL for early and late gain on SSC 9, but these QTL were not confirmed by other studies.

Back fat thickness.

We found 20 significant QTL at the 5% chromosome-wise level for the different traits associated with back fat thickness (Table 3). It should be noted, however, that these traits tend to be highly correlated (Huff-Lonergan et al. 2001). Thus, several of these QTL may have pleiotropic effects. On the other hand, several QTL regions could represent more than one QTL. Multi-trait and multi-QTL analyses will be needed to separate these QTL and their effects. The detected QTL for back fat traits jointly explained from 14% to 24% of the phenotypic variance in the F_2 population (Table 4). Our results indicated that Berkshire

alleles tended to be associated with less fat for QTL on SSC 1, 4, 6, 12, 14, and 18 but were fatter for QTL on SSC 5, 7, and 13 (Table 3). Heterozygotes were leanest for QTL on SSC 1 and 13 and fattest for QTL on SSC 4, 6, 12, and 14.

We found QTL on SSC 1 for tenth rib, last rib, lumbar, and average back fat, but in different regions of the chromosome than the QTL detected by Rohrer and Keele (1998a). We did not detect QTL for backfat on SSC2, for which Nezer et al. (1999) and Jeon et al. (1999) found strong evidence for a paternally expressed QTL for fatness in the region of the IGF2 locus. The IGF2 locus is approximately 5 - 10 cM distal to our first marker on SSC2. De Koning et al. (2000b) also found a paternally expressed QTL for back fat thickness near our second marker for SSC2 We did not detect QTL in this region but only considered Mendelian inheritance. Our results confirmed the existence of QTL for back fat on SSC 4, as reported by Andersson et al. (1994), Marklund et al. (1999), Knott et al. 1998 and Perez-Enciso et al. (2000), but not in the same region of the chromosome.

De Koning et al. (2000b) found two QTL affecting intramuscular fat with maternal and paternal imprinting in the short and long arm of SSC 6, respectively. We also found a QTL for back fat on SSC 6, although our QTL was more to the distal end of the chromosome. Rohrer and Keele (1998a) and Rohrer (2000) also identified QTL and suggestive QTL for back fat measures on Chrs. 5, 8, 9,10, 13, 14, and X. We found QTL in the same regions on SSC 5 for lumbar back fat, and average back fat, and on SSC 13 for tenth rib back fat.

By far the greatest evidence for QTL for back fat in our population was on Chr. 7. The F statistic showed convincing evidence of QTL for all back fat traits over a 80 cM range around the center of SSC 7 (from 40 to 120 cM; Fig. 1E). These results confirm QTL that

have been detected in several studies (Marklund et al. 1999; Moser et al. 1998; Rohrer and Keele 1998a; Walling et al. 1998; Wang et al. 1998; De Koning et al. 1999; Rohrer 2000). While the fatter Meishan breed in these studies had a cryptic allele for leanness on SSC 7, Berkshire alleles were associated with considerably greater fatness in our study (Table 3), as expected from breed differences (Table 1). Recently, Harlizius et al. (2000) reported a QTL for fatness on the X Chr which a Meishan cross. This QTL was not confirmed in our study.

Loin eye area.

We detected two QTL for loin eye area, on SSC 1 and 4, of which one was significant at the 5% genome-wise significance level (Tables 3 and 4). The QTL on SSC 1 confirms results of Rohrer and Keele (1998b), who found a QTL for loin depth on SSC 1 in the same region. Our QTL on SSC 4 was not confirmed by previous studies. Previous studies also reported evidence for QTL for loin depth in exotic crosses on Chrs 2 (Nezer et al. 1999; Jeon et al. 1999), 3 (Andersson-Eklund et al. 1998), 6 (Moser et al.1998), 7 (Rothschild et al. 1995; De Koning et al. 2000a, 2000b), 8 (Andersson-Eklund et al. 1998; Rohrer and Keele 1998b), 9 (De Koning et al. 2000a), 11, 14 (Rohrer and Keele 1998b), 16 (De Koning et al. 2000a), and on the X Chr (Rohrer and Keele, 1998b). We were not able to confirm any of these findings.

Carcass length.

Our results revealed suggestive QTL on SSC 6, 11, and X, with the Berkshire alleles resulting in greater length for two out of three QTL (Table 3). These effects accounted for nearly 10.6% of the variation (Table 4). The QTL on the X Chr was in the same region as a QTL found by Rohrer and Keele (1998b). Other QTL have been reported for carcass length

on SSC 1 (Rohrer and Keele 1998b), SSC 4 (Andersson-Eklund et al. 1998, Rohrer and Keele 1998b), SSC 7 (Rohrer and Keele 1998b), and SSC 8 (Andersson-Eklund et al. 1998; Rohrer and Keele 1998b).

Carcass weight.

The statistical model included a covariable for slaughter weight. Therefore, results for carcass weight reported here reflect an indirect measure of yield or dressing percentage. When interpreting the QTL effect, a difference of 0.5 kg in carcass weight translates into an effect of 0.4% for dressing percentage for a pig of average live weight of 118 kg.

Five QTL were identified for carcass weight (Table 4), on SSC 4, 7, 8, 13, and 14 (Table 3), of which one (on SSC 4) was significant at the 1% genome-wise level. The QTL on SSC 4 was in the same region as the QTL found for last rib back fat (Table 3). Individuals that were homozygous for Berkshire alleles had higher yield or carcass weight than those with Yorkshires alleles for all QTL, except for the QTL on SSC 8 and 13. Four of the five QTL showed high degrees of overdominance. For these QTL, heterozygotes with regard to breed origin had lower yields than either of the homozygotes.

Andersson-Eklund et al. (1998) also reported QTL for carcass weight on SSC 4, 7, and 8, in agreement with this study. Rohrer and Keele (1998b) reported carcass weight QTL on SSC 3 and 7. We also found a QTL for carcass weight on SSC 7 in the same region. Other studies (Moser et al. 1998) have reported effects on SSC 6 when certain alleles of the RYR1 gene were involved, but this was not the case in our families.

Conclusions

Despite limited breed differences, a total of 36 QTL were found to segregate between the Berkshire and Yorkshire breeds for a total of 11 growth and body composition traits, of which 3 and 5 QTL were significant at the 5% and 1% genome-wise levels. These QTL explained from 2.9% to 24.1% of the phenotypic variance for the individual traits in the F_2.

Both breeds had favorable QTL on separate chromosomes for many of the growth and composition traits studied here. There was some evidence on several chromosomes that cryptic alleles existed that favored the breed least expected to have them. Use of these QTL in marker assisted selection could result in substantial improvements.

In this study, we reported QTL significant at the 5% and 1% genome-wise level, as well as those significant at the 5% chromosome-wise level. Although several of these QTL may be false positives, the reporting of QTL at this level of significance is justified by the need to provide other researchers a complete picture of QTL segregating in our family, which will allow them to confirm our results or attempt to identify the individual genes responsible for the traits.

In this study we considered only single QTL models with Mendelian inheritance, with the aim to detect QTL that segregate between the two breeds. Additional statistical analyses to consider multiple QTL, gametic imprinting, and within breed QTL effects is in progress.

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Table 1. Means and standard deviations for traits of interest measured on 525 F_2 animals and expected differences between breed means (Berkshire minus Yorkshire)[a].

Traits analyzed for QTL mapping	Mean	Std Dev	Berk -York[a]
Birth weight (kg)	1.55	0.325	NA[b]
16-Day weight (kg)	4.95	1.311	NA
Average daily gain to weaning (kg/day)	0.24	0.074	0.005
Average daily gain on test (kg/day)	0.69	0.065	0.009
Carcass weight (kg)	87.08	5.733	NA
Carcass length (cm)	84.16	2.454	-1.524
Tenth rib back fat (cm)	3.19	0.779	1.016
Lumbar back fat (cm)	3.58	0.757	1.016
Last rib back fat (cm)	3.16	0.609	0.664
Average back fat (cm)	3.31	0.641	NA
Loin eye area (cm^2)	35.59	5.684	-5.548
Additional Traits:			
Live weight at slaughter (kg)	118.11	6.964	NA
Dressing percent (%)	73.72	1.95	0.0

[a]Expected difference between breed means based on twice the difference observed in crossbreds in the NPPC genetic evaluation program (Goodwin and Burroughs 1995).
[b]NA: Not available.

Table 2. Markers used in the QTL mapping project, their map position based on the F_2 data and information content. Distances are in cM relative to position of the first marker on each chromosome. For comparison, see USDA Map (Rohrer et al. 1996).

Marker	SSC	Position	Number of alleles	IIC[a]	EIC[b]
SW1515	1	0	7	0.97	0.97
SWR2300	1	18.1	3	0.25	0.75
S0008	1	27.4	3	0.90	0.90
S0312	1	42.9	5	0.94	0.94
S0331	1	56.5	5	0.96	0.96
SW974	1	75.5	11	0.92	0.94
SW1301	1	117.6	5	0.80	0.80
SW2623	2	0	5	0.90	0.93
SW2445	2	27.9	4	0.89	0.91
SW766	2	71.3	3	0.73	0.84
SW2157	2	86.3	6	0.89	0.92
SW1408	2	90.1	6	0.44	0.88
SW1844	2	111.6	3	0.72	0.84
SWR308	2	136.9	5	0.86	0.92
S0036	2	143.3	6	0.97	0.97
SW274	3	0	4	0.66	0.77
SW2021	3	19.7	7	0.78	0.83
SW2429	3	31.5	3	0.32	0.71
SW1443	3	58	3	0.33	0.87
S0206	3	60.9	5	0.85	0.89
ACTG2	3	77.6	4	0.78	0.88
SW2408	3	111.9	5	0.95	0.95
SW349	3	128.1	7	0.84	0.92
SW2404	4	0	6	0.89	0.94
SW2509	4	13.7	4	0.55	0.81
S0301	4	33.4	5	0.58	0.75
SW45	4	65.6	4	0.52	0.73
SW512	4	86.4	3	0.62	0.83
SW2435	4	101.9	3	0.60	0.83
SW58	4	110.2	6	0.75	0.89
SW1461	4	130.6	7	0.96	0.96
ACR	5	0	4	0.48	0.88
SW413	5	2.2	5	0.80	0.90
SW1482	5	29.6	8	0.84	0.88
SW2	5	61.8	5	0.44	0.68
SW904	5	86.1	5	0.93	0.95

[a]IIC: Information content based on data for this marker only.
[b]EIC: Effective information content including information on linked markers

Table 2. (Continued)

Marker	SSC	Position	Number of alleles	IIC[a]	EIC[b]
SW995	5	102.1	5	0.73	0.88
SW378	5	113.9	3	0.62	0.81
SW2535	6	0	3	0.59	0.82
SW2406	6	12.1	5	0.92	0.95
SW1038	6	40.2	3	0.58	0.81
SWR1130	6	53.6	7	0.84	0.91
SW122	6	66.2	6	0.95	0.97
SW1059	6	78.3	8	0.88	0.93
DG93	6	96.5	6	0.74	0.84
SW322	6	119.8	5	0.90	0.92
SW2052	6	142.9	6	0.97	0.97
S0025	7	0	4	0.87	0.91
S0064	7	28.9	5	0.61	0.79
TNFB	7	48.3	8	0.83	0.91
SWR1928	7	64.2	4	0.76	0.88
SW252	7	83	4	0.90	0.93
SW1083	7	95.6	2	0.30	0.76
S0101	7	116.9	4	0.87	0.91
SW764	7	139.1	4	0.93	0.95
S0098	8	0	4	0.55	0.71
SWR1101	8	25.4	7	0.81	0.88
S0086	8	48.2	4	0.77	0.90
SW2160	8	59.9	5	0.97	0.97
SW1551	8	75.7	3	0.46	0.77
SPP1	8	99.8	7	0.84	0.91
S0178	8	115.9	4	0.97	0.97
SWR68	9	0	2	0.05	0.51
SW21	9	14.1	4	0.58	0.71
SW911	9	37	3	0.66	0.78
SW827	9	50.8	3	0.57	0.74
SW1491	9	76.5	3	0.49	0.75
SW2093	9	94.5	5	0.80	0.82
SW2116	9	116.4	3	0.67	0.77
SW1349	9	143.3	4	0.52	0.64
SWR136	10	0	5	0.57	0.70
SW443	10	18.2	5	0.50	0.74
SW2491	10	38.8	4	0.83	0.89
SWR198	10	56.1	5	0.97	0.97
SWR493	10	79.2	3	0.54	0.77
SW1626	10	102.8	6	0.92	0.92
SW2067	10	120.4	6	0.88	0.92
S0385	11	0	5	0.74	0.81
SW1632	11	18	4	0.40	0.69
S0071	11	45.1	5	0.92	0.93
SW13	11	85.8	5	0.83	0.83
S0229	12	0	6	0.96	0.97
SW874	12	34.1	7	0.97	0.97

Table 2. (Continued)

Marker	SSC	Position	Number of alleles	IIC[a]	EIC[b]
S0090	12	46.9	5	0.69	0.84
S0147	12	61.9	4	0.79	0.88
SW2180	12	90.4	4	0.69	0.77
SWR1941	13	0	5	0.70	0.84
SW1407	13	20.8	6	0.97	0.97
SW344	13	32.3	5	0.97	0.97
S0068	13	45.9	5	0.95	0.96
SW398	13	58	5	0.97	0.98
SW1056	13	73.7	4	0.41	0.73
SW2097	13	98.8	3	0.81	0.83
SW857	14	0	5	0.62	0.82
SW1027	14	16.4	7	0.92	0.94
SWR84	14	38.2	4	0.97	0.97
S0007	14	46.2	8	0.95	0.96
SW77	14	57	5	0.97	0.97
SW55	14	70.4	5	0.85	0.92
SWC27	14	110.3	5	0.58	0.64
SW1416	15	0	5	0.97	0.98
S0148	15	21.5	5	0.89	0.93
SW964	15	38.2	5	0.86	0.92
SW1683	15	59.3	4	0.70	0.88
SW936	15	69.1	4	0.76	0.91
SW1983	15	80.5	7	0.90	0.94
SW1119	15	96	5	0.61	0.83
SW2411	16	0	5	0.76	0.82
SW2517	16	31.8	4	0.95	0.95
S0105	16	58.4	5	0.96	0.96
SW335	17	0	5	0.96	0.97
SWR1004	17	7	5	0.94	0.97
S0292	17	48.5	5	0.80	0.89
S0359	17	59	4	0.76	0.90
S0332	17	82.1	4	0.92	0.95
SW2427	17	94.4	7	0.44	0.80
SW1023	18	0	5	0.86	0.92
SW1984	18	21.1	5	0.91	0.94
S0062	18	32.1	4	0.56	0.83
S0177	18	59.4	6	0.96	0.96
SW949	X	0	6	0.84	0.84
SW1903	X	54.9	4	0.68	0.93
SW2126	X	55.1	4	0.83	0.96
SW1943	X	74.9	4	0.96	0.96
SW2588	X	96.8	3	0.96	0.96

Table 3. Evidence for QTL significant at the 5% chromosome-wise level for various growth and composition traits by chromosome. Estimated significance levels (F value), location, gene effects and % of F_2 variance explained by each QTL.

SSC	Trait	F-value[a]	Location (cM)	Additive Effect	Additive S.E.	Dominance Effect	Dominance S.E.	% variance[c]
1	Average back fat (cm)	6.79	29	-0.09	0.03	-0.12	0.05	2.83
1	Tenth rib back fat (cm)	11.32**	29	-0.11	0.04	-0.23	0.06	4.78
1	Last rib back fat (cm)	6.61	66	-0.13	0.04	-0.01	0.06	3.03
1	Lumbar back fat(cm)	6.96	64	-0.15	0.44	-0.08	0.07	3.12
1	Loin eye area (cm2)	10.34**	29	1.11	0.31	1.33	0.47	4.21
2	Average daily gain on test (kg/day)	8.31*	87	0.015	0.00	0.010	0.006	3.65
3	Birth weight (kg)	5.20	19	-0.02	0.020	-0.09	0.03	2.88
4	Average daily gain on test (kg/day)	8.87*	97	-0.006	0.004	0.03	0.007	5.71
4	Carcass weight (kg)	11.76**	123	0.71	0.16	0.41	0.25	5.97
4	Loin eye area (cm2)	7.87	92	1.38	0.36	-0.64	0.59	4.20
4	Last rib back fat (cm)	5.86	101	-0.03	0.04	0.19	0.07	3.18
4	Lumbar back fat(cm)	5.29	107	-0.03	0.04	0.27	0.07	2.92
5	Average back fat (cm)	7.35	113	0.15	0.04	-0.002	0.06	3.75
5	Last rib back fat (cm)	9.51*	113	0.17	0.04	0.04	0.06	4.83
5	Lumbar back fat(cm)	7.25	107	0.17	0.05	0.11	0.07	3.79
6	Tenth rib back fat (cm)	6.14	128	-0.14	0.05	0.15	0.08	3.63

[a]Chromosome-wise F-statistic thresholds at the 5% level, as determined by permutation test were as follows: (1) 5.08, (2) 5.12, (3) 5.14, (4) 5.14, (5) 4.99, (6) 5.32, (7) 5.25, (8) 5.03, (9) 5.09, (10) 5.11, (11) 4.59, (12) 4.78, (13) 5.03, (14) 5.02, (15) 5.02, (16) 4.34, (17) 4.86, (18) 4.45, (X) 4.80. [b]Additive (a) and dominance (d) QTL effects correspond to genotype values of +a, d, and –a for, respectively, individuals having inherited two Berkshire alleles, heterozygotes, and individuals with two Yorkshire alleles. Positive additive effects indicate that Berkshire alleles increased the trait, negative that the Berkshire alleles decreased it. Dominance effects are relative to the mean of the two homozygotes.

[c] % variance = genetic variance at the QTL based on estimated additive and dominance effects and allele frequencies of ½, as a percentage of the residual variance in the F_2.

* Significant at the 5% genome-wise level (F>8.22)

** Significant at the 1% genome-wise level (F> 9.96)

Table 3. (Continued)

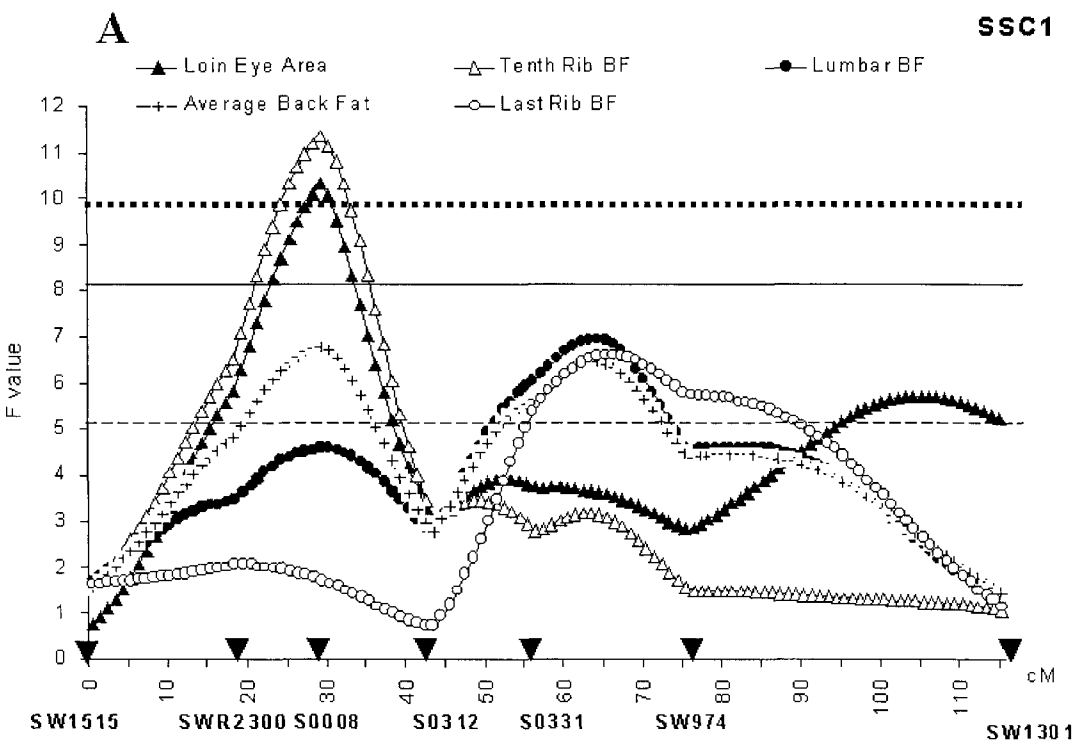
SSC	Trait	F-value[a]	Location (cM)	Additive Effect	Additive S.E.	Dominance Effect	Dominance S.E.	% Variance
6	Carcass length (cm)	5.44	141	0.46	0.14	0.05	0.19	2.59
7	Average back fat (cm)	11.10**	58	0.17	0.04	0.05	0.06	5.34
7	Lumbar back fat (cm)	13.81**	72	0.24	0.05	-0.07	0.08	6.88
7	Tenth rib back fat (cm)	5.60	58	0.14	0.05	0.09	0.07	2.83
7	Last rib back fat (cm)	7.27	74	0.14	0.04	-0.04	0.06	3.69
7	Carcass weight (kg)	7.69	95	0.41	0.16	-0.77	0.26	4.68
8	Average daily gain on test (kg/day)	6.28	48	-0.014	0.004	0.005	0.006	2.76
8	Carcass weight (kg)	7.33	48	-0.34	0.15	0.67	0.21	3.36
9	Average daily gain to weaning (kg/day)	6.38	37	0.008	0.005	0.023	0.007	3.66
9	Average daily gain on test (kg/day)	5.32	116	0.014	0.004	-0.002	0.007	2.86
11	Carcass length (cm)	5.72	13	-0.36	0.15	0.60	0.27	4.06
12	Last rib back fat (cm)	4.78	81	-0.14	0.05	-0.12	0.08	4.52
13	Average back fat (cm)	5.84	27	0.09	0.04	-0.13	0.05	2.81
13	Tenth rib back fat (cm)	7.08	23	0.12	0.04	-0.14	0.06	3.05
13	Last rib back fat (cm)	5.35	36	0.07	0.04	-0.15	0.05	2.69
13	Carcass weight (kg)	5.52	54	-0.19	0.15	-0.67	0.22	2.61
14	Last Rib back fat (cm)	5.29	57	-0.04	0.03	0.14	0.05	2.09
14	Carcass weight (kg)	5.51	58	0.19	0.14	0.62	0.20	2.30
18	Average back fat (cm)	4.46	5	-0.12	0.04	0.02	0.06	2.33
X	Carcass length (cm)	5.17	75	0.55	0.17	-0.02	0.18	3.95

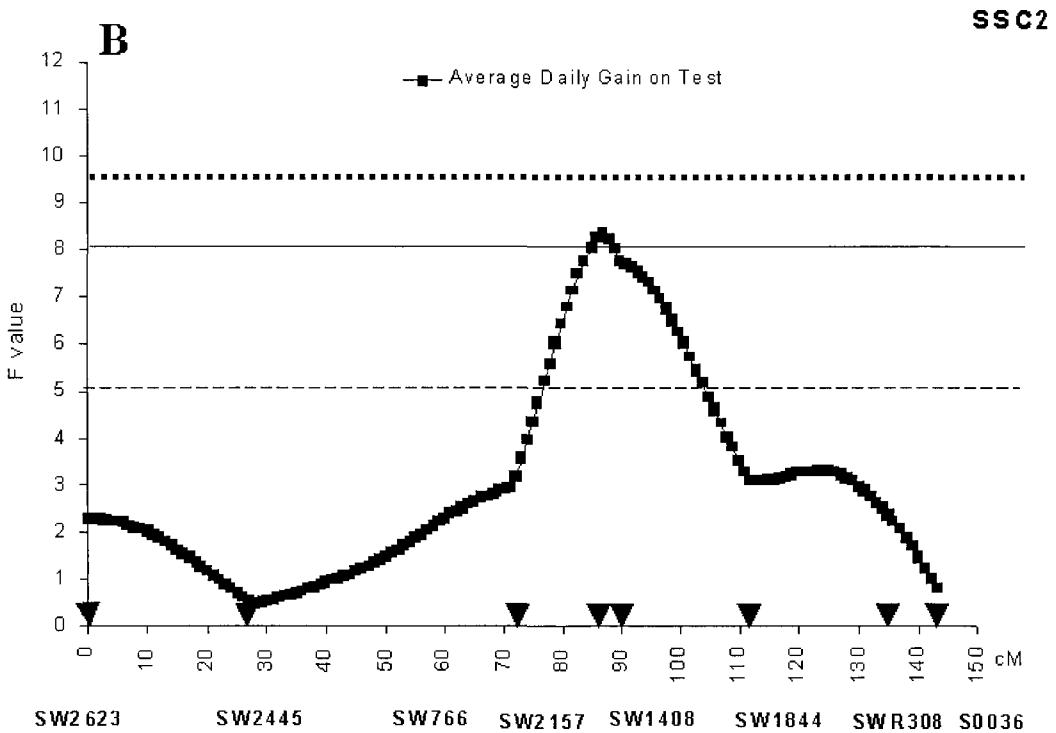
Table 4. Summary of QTL significant at the 5% chromosome-wise level (%5 chr), the 5% genome-wise level (%5 gen) (F>8.22) and the 1% genome-wise level (%1 gen) (F>9.96) by trait.

Trait	No. of significant QTL %5 chr	%5 gen	%1 gen	% of F_2 variance explained[a]
Birth weight	1			2.9
Average daily gain to weaning	1			3.7
Average daily gain on test	2	2		15.0
Tenth rib back fat	3		1	14.3
Lumbar back fat	3		1	16.7
Last rib back fat	6	1		24.1
Average back fat	4		1	17.0
Loin eye area	1		1	8.4
Carcass length	3			10.6
Carcass weight	4		1	19.0

[a]The real variance could be higher, because we did not account for multiple QTL in adjacent intervals.

Figure 1. F-ratio curves for evidence of QTL. The x-axis indicates the relative position on the linkage map. The y-axis represents the F-ratio. Arrows on the x-axis indicate the position where a marker was present. Three lines are provided for 5% chromosome-wise (------), 5% genome-wise (_____) and the 1% genome-wise (.....) significance.

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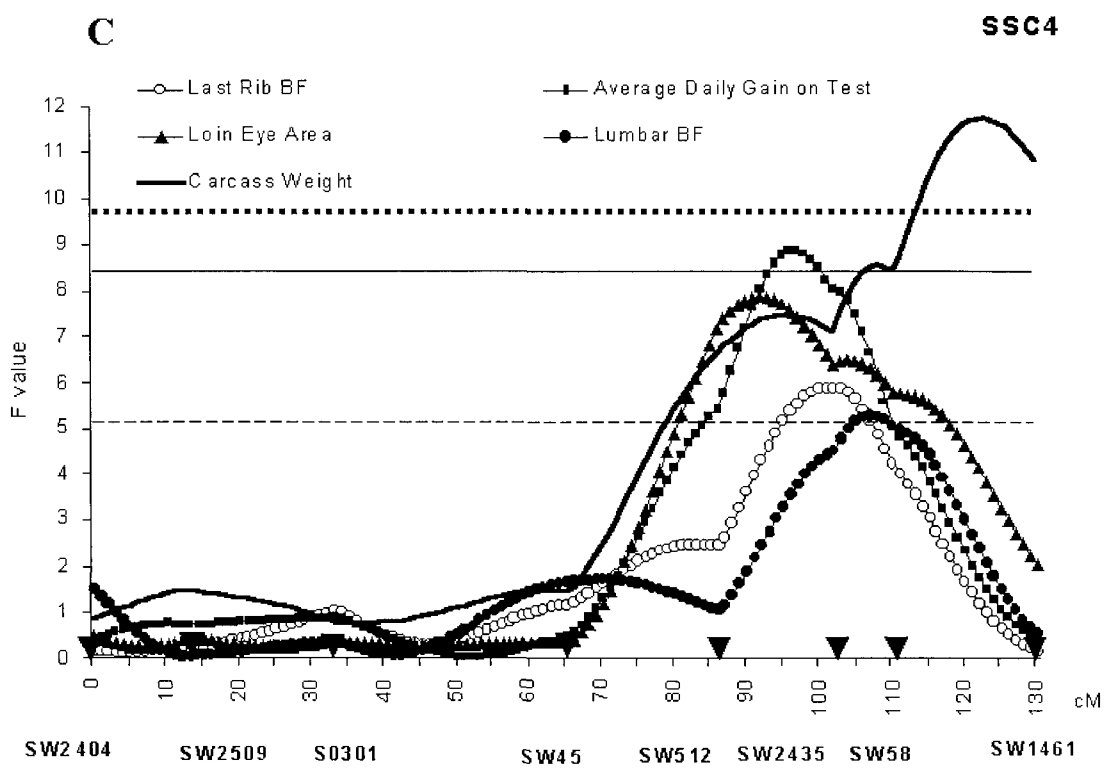
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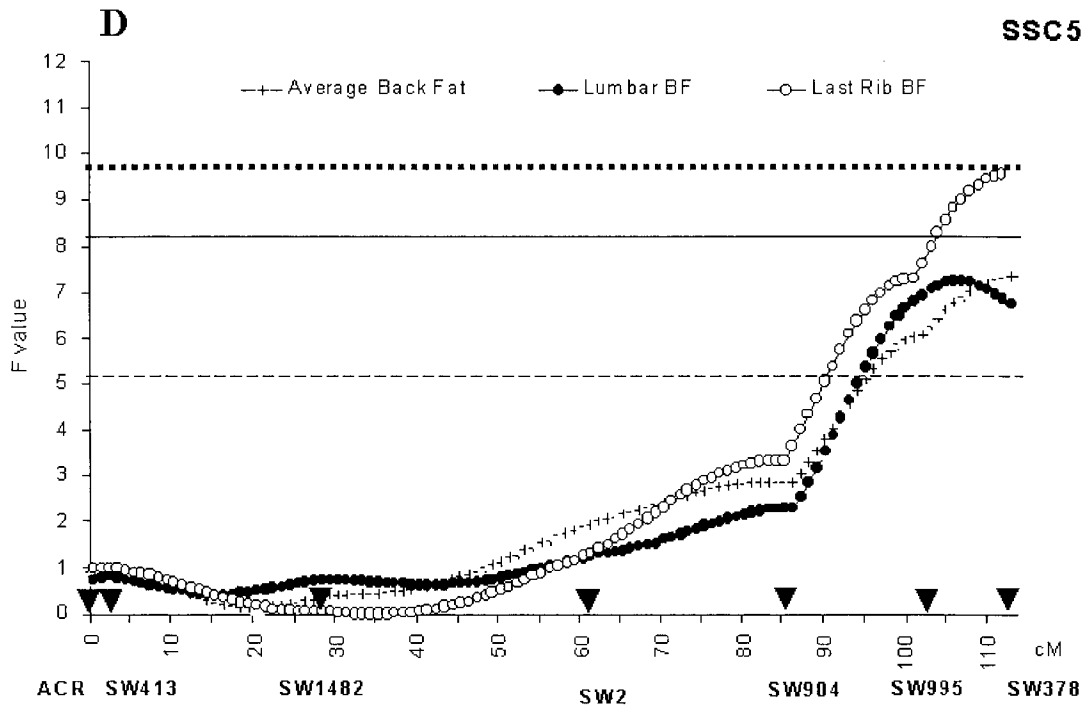
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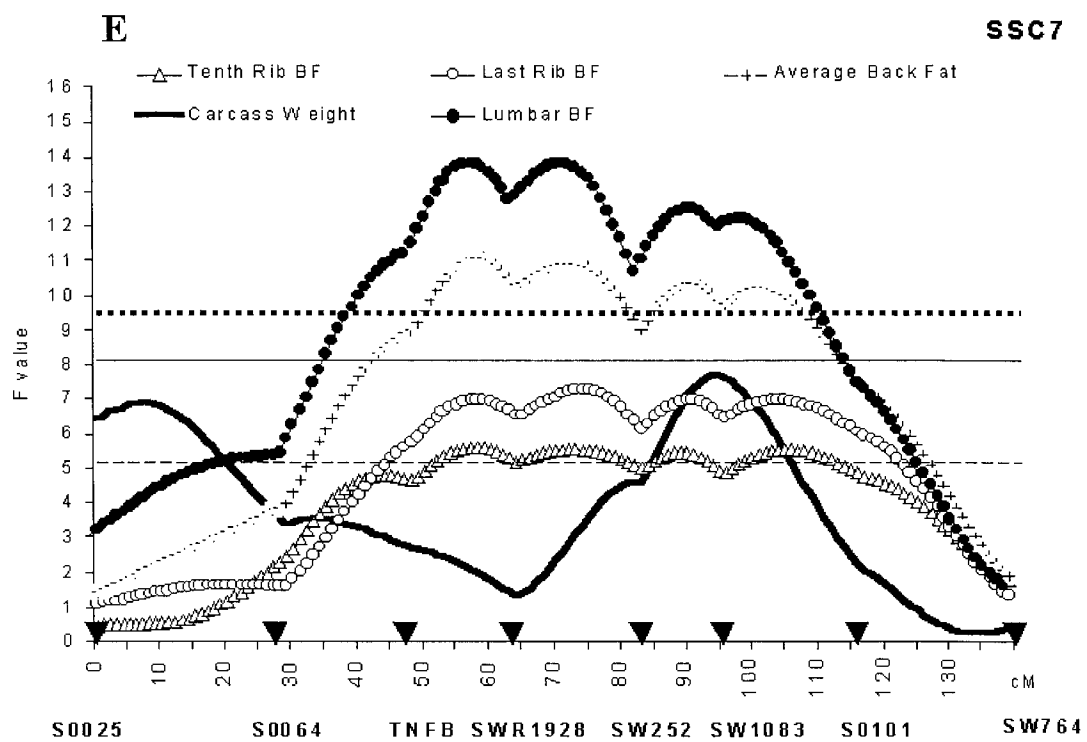
SW995
SW378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SW252
SW1083
S0101
SW764

CHAPTER 3. A MOLECULAR GENOME SCAN ANALYSIS TO IDENTIFY CHROMOSOMAL REGIONS INFLUENCING ECONOMIC TRAITS IN THE PIG. II. MEAT AND MUSCLE COMPOSITION.

A paper published in Mammalian Genome[1]

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Abstract

Molecular genetic markers can be used to identify chromosomal regions that contain quantitative trait loci (QTL) for meat quality and muscle composition traits in farm animals. To study this in pigs, a resource family was generated from a cross between two Berkshire grand sires and nine Yorkshire grand dams. A total of 525 F2 progeny from 65 matings of F1 parents were produced. Phenotypic data on 28 meat quality traits (drip loss, water holding capacity, firmness, color, marbling, percent cholesterol, ultimate pH, fiber type, and several sensory panel and cooking traits) were collected on the F2 animals. Animals were genotyped for 125 microsatellite markers covering the entire genome. Least squares regression interval mapping was used for QTL detection. Significance thresholds were determined by permutation tests. A total of 60 QTL were detected at the 5% chromosome-wise level for meat quality traits, on chromosomes 1, 2, 4, 5, 6, 8, 10, 11, 12, 13, 14, 15, 17, 18, and X, of which 9 and 1 QTL were significant at the 5% and 1% genome-wise levels (on chromosomes 1, 5, 12, 15, and 17), respectively.

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Introduction

Pork quality comprises a set of key fresh meat quality, processing and sensory characteristics that are important for the future profitability and competitiveness of the swine industry. These include intramuscular fat, cholesterol, ultimate pH, color, water holding capacity or drip loss, tenderness, cooking loss and sensory traits involving taste (Sellier, 1998). In the past, leanness was considered one of the most important traits. As a result, dramatic improvements in the body composition of pigs have been made. However, it has been shown that lean meat is not always associated with good meat quality (Cameron, 1990; Hovenier et al. 1992) and therefore several other traits must be considered to improve pork quality. Improving meat quality genetically is difficult by standard selection methods but possible if the genes responsible for meat quality are identified and mapped.

A limited number of studies have attempted to map QTL for meat quality traits but they have generally involved a cross with at least one exotic breed (Andersson-Eklund et al. 1998; Milan et al. 1998; Wang et al.1998; Moser et al. 1998; Yu et al. 1999; De Koning et al. 2000a, 2000 b). These studies have reported the existence of QTL for meat quality traits on almost all chromosomes, except 10, 17, and 18. These QTL must be confirmed in other crosses, in particular those involving breeds that are of commercial (economic) interest.

The Berkshire and Yorkshire are breeds of commercial interest that have demonstrated considerable differences in meat quality, with Berkshire pigs having very positive meat quality traits (Goodwin and Burroughs, 1995). In order to identify the chromosomal regions and genes responsible for differences in meat quality traits in these

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breeds, a three generation resource family was developed. Malek et al. (2001) reported the identification of several QTL for growth and body composition traits in this population and Huff-Lonergan et al. (2001) described relationships among meat quality traits. The objectives of this study were to analyze this resource family for QTL for muscle and meat quality traits. This study represents the first genome-wise QTL scan for meat quality traits using both of these commercial breeds.

Material and Methods Management

Family structure and Management.

A three-generation resource family was developed using two purebred Berkshire grand sires (Casino and Count) and nine Yorkshire grand dams. Details on the family structure and management of the pigs are in Malek et al. (2001).

Traits measured.

Phenotypic data for a total of 28 meat quality traits were collected on the F2 animals. Traits measured are listed in Table 1. Measurements were taken primarily at two locations: at the Hormel slaughter plant in Austin, Minnesota at 24 hrs after slaughter and at the Iowa Sate University Meat Laboratory in Ames 48 hrs after slaughter. All measurements were taken by trained personnel following the guidelines of the National Pork Producers Council (NPPC, 1991).

Carcass traits evaluated at the slaughter plant after slaughter and chilling included the subjective quality traits of marbling, firmness, and color in the loin. Subjective traits were scored on a scale from 1 to 5, with higher values indicating greater marbling, greater

firmness, and darker color. Objective measurements of color were taken with a Minolta chromometer and a Hunter lab scan. Minolta and Hunter L values measure light reflectance of the muscle. Lower values indicate darker color, which is desirable, and higher values indicate paler, lighter colored meat. Muscle pH was measured in the longissimus dorsi and the semimembranosus muscles at 24 hours after slaughter, using a glass penetration pH electrode. Measurement of Minolta and Hunter L values and pH was repeated at 48 hours postmortem in the Ames laboratory.

Two measures of the ability of the muscle to retain moisture, drip loss and water holding capacity, were taken. Drip loss measures the amount of moisture (purge) lost from the product over a period of time. Water holding capacity is a complementary measure of the ability of meat to retain water. Drip loss was measured on a size-standardized sample from the longissimus dorsi (3 cm in diameter and 2.5 cm thick) (Honikel et al. 1986; Kauffman et al. 1986) that was collected at 48 hours postmortem. The sample was weighed, suspended in a plastic bag, held at 4°C for 72 hours, and re-weighed at the end of the holding time. Drip loss was calculated as the percentage of product weight that was lost over the 72 hour storage period. This was done with duplicate samples and the average value was used for analysis. Water holding capacity was measured using the filter paper press method (Kauffman et al. 1986), which evaluates the amount of moisture lost from the surface of the loin shortly after cutting. A pre-weighed piece of filter paper, which was exposed to the atmosphere for 10 minutes, was placed on a fresh cut of the loin muscle 48 hours postmortem for three seconds to allow it to absorb surface moisture, and then re-weighed. The difference in weight was used as the measure of water holding capacity (Kauffman et al. 1986), with a lower value indicating that less moisture was lost from the tissue, which is more desirable.

At 48 hours postmortem, a sub-sample of the loin was frozen and sent to the University of Illinois, where glycogen, free glucose, glucose-6-P, and lactate content were measured in μMol/g (Monin and Sellier, 1985). Postmortem metabolism of elevated glycogen stores results in increased production of lactate, which is a pH lowering by-product of muscle metabolism. Glycolytic potential is a measure of glycogen stores and was calculated as follows: glycolytic potential = 2 x ([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate] (Monin and Sellier, 1985; Maribo et al. 1999). Glycolytic potential is expressed in μMol lactate equivalents per gram muscle wet weight. In addition to glycolytic potential and lactate concentration, residual glycogen concentration was used as a trait of interest in this study. Residual glycogen is the glycogen remaining in the muscle that was not converted to lactate and glucose-6-phosphate.

Total lipid in the longissimus dorsi was measured as described by Bligh and Dyer (1959) and expressed as a percentage of tissue weight. Total lipids were then dissolved in isopropanol and assayed for concentration of total cholesterol using an enzymatic procedure (Sigma Cholesterol Kit No. 352, Sigma Chemical Co., St. Louis, MO). Cholesterol was reported in mg per 100 g of tissue.

Muscle fiber type composition was evaluated in 48-hour postmortem samples from the longissimus dorsi by separation of myosin isoforms on high porosity SDS-PAGE gels. The procedure used was as described by Talmadge and Roy (1993) but with modifications as described by Huff-Lonergan et al. (2001). Results were expressed as the ratio of the density of the IIa band of myosin to the density of the IIb band within a sample. Porcine diaphragm muscle (extracted as described in Huff-Lonergan et al. 2001) was used as a standard on each

gel to aid in identifying the myosin isoforms. Diaphragm muscle contains primarily type IIa, IIx, and type I associated myosin isoforms (Talmadge and Roy, 1993).

To evaluate the sensory characteristics of the meat, vacuum packaged boneless chops from the longissimus dorsi of each animal were taken 48 hours after slaughter and stored for 10 days at 4°C. Following the storage period, chops were broiled to 71°C in an electric oven broiler (Amana Model ARE 60) that had been preheated to 210°C. The temperature of each chop was monitored in the center of the chop using thermocouples (Chromega/Alomega) attached to an Omega digital thermometer (Model DSS-650, Omega Engineering). Cooking loss was calculated from weights taken before and after broiling and was expressed as a percentage. Instrumental measurement of tenderness of the broiled chops was evaluated using a circular five-pointed star-probe (9 mm in diameter with 6 mm between each point) attached to an Instron Universal Testing Machine (Model 1122). A 100 kg load cell was used with a crosshead speed of 200 mm/min. The star-probe attachment was used to determine the amount of force needed to puncture and compress the chop to 80% of the sample height. Each chop was punctured 3 times and the average was recorded.

Sensory evaluation of the broiled chops was done using three highly-trained professional sensory panelists. Panelists were seated in individual booths with red lighting overhead to mask any differences in product color. Cubes, 1.3 cm in size, were removed from the center of the broiled loin chops, placed in preheated, individually-coded glass petri dishes and served to each panelist. Room temperature deionized, distilled water and unsalted crackers were used to cleanse the palates of the panelists between samples. Samples were evaluated for degree of juiciness, tenderness, chewiness, pork flavor, and off-flavor using a 10-point category scale. The scale was anchored on the left end with a term representing a

low degree of juiciness, tenderness, chewiness, flavor, and off-flavor intensity. On the right end of the scale was a term representing a high degree of each characteristic. Any flavor that was not associated with normal pork flavor was considered as an off-flavor. The values for each pork chop were averaged across the three panelists.

DNA isolation, marker selection and genotyping.

Usual methods were used to collect samples and isolate DNA. All animals were genotyped for 125 markers as described by Malek et al. (2001). Of the original 525 F2 animals, likely parentage (or collection) problems existed for 13 F2 animals and these were removed, leaving 512 animals for analysis.

QTL analyses.

Marker linkage maps and the QTL analyses used are explained in Malek et al. (2001). Significance levels at the 5% chromosome-wise and at the 5% and 1% genome-wise levels were determined by permutation as described by Malek et al. (2001).

Results and Discussion

Arithmetic means and standard deviations measured for each trait on the F2 animals are listed in Table 1. Measurements were not available for all traits on all animals due to occasional sampling problems. Results conformed to the usual range of measurement scores. Relationships among traits are described in Huff-Lonergan et al. (2001). Expected differences between the Berkshire and Yorkshire breeds for the traits evaluated are also in

Table 1. Breed differences are based on crossbred results from the National Pork Producer Council's Genetic Evaluation Program (Goodwin and Burroughs, 1995).

QTL results.

Results for QTL that were detected at the 5% chromosome-wise level are in Table 2 and summarized by trait in Table 3. For the pig genome, the 5% chromosome-wise significance level roughly corresponds to the genome-wise suggestive level (Lander and Kruglyak, 1995; De Koning et al. 1999). The QTL graphs for chromosomes with evidence for QTL at the 5 and 1% genome-wise levels are presented in Figure 1. To avoid double counting, cases where evidence for QTL extended over multiple adjacent marker intervals were reported as a single QTL in Tables 2 and 3. Further dissection of these QTL will require additional statistical analyses.

A total of 60 QTL were detected at the 5% chromosome-wise level for the 28 traits evaluated (Table 2), of which 9 were significant at the 5% genome-wise level. One QTL, for Hormel Loin pH on chromosome 15 (Fig. 1G), was significant at the 1% genome-wise level. Thus, substantially more QTL were detected for the 28 traits evaluated than the 28, 1.4, and 0.3 QTL that would be expected at the suggestive, 5%, and 1% genome-wise levels by chance alone. Significant QTL were detected for nearly all traits and on all chromosomes, except on chromosomes 3, 9, and 16. Most QTL accounted for 2% to 5% of the F2 variance but one reached 10% (color score on SSC 12). As noted by Malek et al. (2001), variance accounted by individual QTL (Table 2) may be overestimated. However, total variances explained by QTL by trait, as reported in Table 3, may be underestimated because existence of multiple QTL in adjacent marker intervals was ignored. Both breeds had favorable QTL

alleles on separate chromosomes, despite Berkshires having more desirable meat quality breed characteristics for most traits (Table 1). In the following presentation, QTL detected will be discussed on a trait basis and related to literature findings.

Color and light reflectance.

Color is one of the most important visual parameters for meat quality. Color determines initial acceptance or rejection in the marketplace. Lighter colored pork is often associated with more drip loss, poorer water holding capacity, and lower pH. Huff-Lonergan et al. (2001) also found significant negative correlations of ultimate pH with Hunter L values and drip loss in our F2 population.

Three QTL were found for subjective color in this study (Table 3), of which two were significant at the 5% genome-wise level (Fig. 1E, and I). A total of 19 QTL were detected for the subjective and objective measurements related to color, of which 4 were significant at the 5% genome-wise level (Tables 2 and 3). Because of the relationships between these traits, several QTL likely represent a single QTL with pleiotropic effects.

There was suggestive evidence of QTL for four color traits, including subjective color, within a 60 cM region of SSC2 (Table 2). Two and three objective reflectance traits, respectively, showed QTL at the same positions on SSC4 and SSC5. A QTL for subjective color was detected on SSC12 at the 5% genome-wise level, but this QTL was not supported by QTL for reflectance traits. The phenotypic correlation of subjective color with Lab Loin Hunter was –0.7 in this population (Huff-Lonergan et al. 2001), thus this could represent a QTL subjective color that is not pleiotropic for reflectance traits. Chromosome 15 showed QTL for three reflectance traits within a 30 cM region. Chromosome 17 had the greatest

evidence for QTL, with significance at the 5% genome-wise level at the same position for three traits, including subjective color.

For two of the three QTL for subjective color, Berkshire alleles were associated with lighter colored meat than were Yorkshire alleles (Table 2). Berkshire alleles were associated with better (lower) reflectance scores on SSC 14, 15, and 17, but with higher reflectance on SSC 2, 4, 5, 7, and 18.

Andersson-Eklund et al. (1998) found some evidence that the proportion of Wild Boar alleles on chromosomes 2, 10, 12, and 15 was associated with QTL affecting meat color in a cross between the Wild Boar and Large White breeds, although no QTL reached genome-wise significance. Wang et al. (1998) reported suggestive QTL on SSC 4 and SSC 7 affecting color. These QTL were significant in individual Chinese by Western breed crosses but not pooled over all crosses evaluated in their study. Jeon et al. (1999) reported a paternally inherited QTL for reflectance on SSC 2 but at the beginning of the chromosome, near IGF-2, not at the distal end as in our study. We did, however, not test for imprinted QTL. De Koning et al (2000a) found a total of nine suggestive and three significant QTL at the genome-wise level for various measures of reflectance: five QTL, on chromosomes 1, 3, 4, 13, and 14 affecting Color-L (lightness, which is the same as Hunter or Minolta of our study). In addition they found four QTL affecting Color-A (green to redness), on chromosomes 3, 13, 14, and 15, and 3 QTL affecting Color-B (blue to yellowness), on chromosomes 4, 13, and 14. They reported that the QTL for color found by Wang et al. (1998) on SSC 4 was at approximately the same position as a significant QTL affecting Color-B in their study. For Color-L, which was the only trait in common with our study, our

QTL on SSC 14 for Hormel Ham Hunter was on a different region of the chromosome than the QTL found by De Koning et al (2000a).

Tissue quality and water holding capacity.

Water holding capacity and drip loss measure the ability of the muscle to retain moisture. Less moisture loss prior to cooking is also often associated with better color, greater firmness and higher pH, which was substantiated by moderately high (0.2 to 0.3) correlations observed in our data (Huff-Lonergan et al. 2001).

Our results found seven significant QTL at the 5% chromosome-wise significance level for water holding capacity and drip loss, on chromosomes 1, 2, 11, and 13 (Table 2). Multiple peaks were found for drip loss and water holding capacity on SSC 2 (Fig. 1B) and for drip loss on SSC 11 (not shown). These peaks may be due to multiple QTL on those chromosomes. Yorkshire alleles were associated with more desirable quality for all QTL related to moisture loss, except for the QTL for drip loss on SSC 1. Based on breed means (Table 1), Yorkshires are expected to have more drip loss.

Andersson-Eklund et al. (1998) reported QTL on chromosomes 1, 2, and 12 affecting drip loss and on chromosomes 12, 13, and 18 for water holding capacity but none reached genome-wise significance. However, they found that the average proportion of wild boar alleles across the genome had highly significant effects on drip loss. De Koning et al. (2000a, b) detected four QTL for drip loss, some with imprinted effects, on chromosomes 4, 6 (maternal), 14 (Mendelian), and 18 (paternal). We were not able to confirm the QTL found by Andersson-Eklund et al. (1998) and De Koning et al. (2000a, b).

Firmness and fiber type could also be considered part of pre cooked tissue quality. Huff-Lonergan et al. (2001) found that fiber type II ratio was negatively correlated with Hunter L values and drip loss in our F2 population, although the magnitude of the relationships was not high (-0.10). Our analyses found QTL for the subjective measure of firmness on SSC 2 and for fiber type on SSC 8. Andersson-Eklund et al. (1998) reported that the proportion of Wild Boar alleles on SSC 2 in their Wild Boar by Large White cross was associated with sarcoplasmic protein extractability, which may be associated with firmness. Milan et al. (1998) reported QTL for muscle fiber type differences on SSC 3, but this QTL was not confirmed in our study.

Fat content.

Visual (subjective) marbling scores correspond to intramuscular lipid content, with a correlation of 0.57 in this F2 population (Huff-Lonergan et al. 2001). Higher lipid content is generally considered more desirable as it adds to flavor and cooking properties and improves tenderness, although correlations between these traits were not very high in our data (<0.25, Huff-Lonergan et al. 2001).

Strongest evidence for a QTL for intramuscular fat was on SSC 1, which showed a QTL for marbling score at the 5% genome-wise level and a suggestive QTL for lipid percent in the same region. (Table 2, Fig. 1A). Berkshire alleles were associated with less intramuscular fat (unfavorable) for both QTL, which is opposite to expectations based on breed means (Table 1). Two additional QTL were found for marbling on chromosomes 8 and 10, both at the suggestive level (Table 2). Again, Yorkshire alleles were superior to

Berkshire alleles. Only one QTL was found for cholesterol concentration (on SSC 18). This QTL was not related to QTL for lipid % or marbling.

De Koning et al (2000a, b) found six QTL for intramuscular fat, with different types of gene expression. These were on chromosomes 4, 6 (maternally inherited), 6 (paternal), 8 (sex specific), 13 (maternal), and the X chromosome. The *H-FABP* gene, a candidate gene associated with fat levels, is known to map to SSC 6 (Gerbens et al. 1997). Yu et al. (1999) also reported a suggestive QTL for marbling on SSC 13. We were not able to confirm any of these QTL in our cross and our evidence for a QTL on SSC 1 was not corroborated by other studies.

Measures of pH.

Ultimate pH is the most commonly used trait to assess pork quality and usually is measured at 24 and 48 hrs post mortem. Ultimate pH of pork is not a direct measure of quality, but it is correlated to the quality traits of color, drip loss, and water holding capacity. Muscle pH post mortem is also correlated with sensory panel traits such as tenderness and juiciness. A higher level of acidity within the muscle (lower pH) causes muscle proteins to denature and lose their ability to hold water. Therefore, meat with higher pH will tend to have more desirable characteristics such as darker color, less drip loss, more firmness, and higher tenderness. In our data, correlations of ultimate pH with measures of water holding capacity, color, and sensory quality were moderately high (0.15 to 0.35) (Huff-Lonergan et al. 2001).

Seven QTL were detected for pH related traits at the 5% chromosome-wise significance level (Table 3), of which three were significant at the 5% genome-wise level (on chromosomes 5 and 15) and 1 at the 1% genome-wise level (on SSC 15). Yorkshire alleles

had higher (better) pH for the QTL on SSC 5, 6, and 14, but Berkshire alleles were better for the QTL on SSC 15.

Two QTL for pH were detected in SSC 5, both for pH in the loin but measured at different times (Table 2, Fig. 1D). The QTL for pH at 24 hours was significant at the 5% genome-wise level and at the distal part of the chromosome, where also QTL were found for three reflectance traits. These likely represent the same QTL, for which Yorkshire alleles were desirable (higher pH and lower reflectance). Other studies have not detected QTL for pH or reflectance on SSC 5.

A suggestive QTL for 24 hour pH in the ham was found on SSC 6 (Table 2). This QTL was near the HAL gene (Fujii et al. 1991), although the positive (detrimental) HAL allele was not present in our population. Gelderman et al. (1996) also demonstrated a QTL for pH on SSC 6 near the HAL gene but using HAL positive pigs. Our results, however, suggest that some mutation other than the well-known detrimental allele might be present in HAL or another closely linked gene.

The suggestive QTL for 24-hour pH in the ham on chromosome 14 (Table 2) was in the same location as a paternally imprinted QTL that was found by De Koning et al (2000a, b). Additional analyses are needed to determine whether our QTL is also subject to imprinting effects. De Koning et al. (2000a, b) also found another QTL on SSC 14 affecting pH, which showed significant differences in estimated QTL effects between sexes. We were not able to confirm this QTL.

Strongest evidence for QTL for pH was on SSC 15 (Table 2, Fig.1G), and which showed QTL in the central and distal regions of the chromosome for three pH measures. These QTL were significant at the 5% genome-wise level for two traits and significant at the

1% genome-wise level for Hormel Loin pH. These QTL were in the same region as QTL for reflectance, glycolytic potential, and sensory traits, and will be discussed in greater detail in the following.

De Koning et al. (2000a, b) also found QTL affecting pH on SSC 4, 9, 11, 18, and X, with a variety of modes of gene expression. We were not able to confirm these results.

Glycolytic potential.

During the first 6 to 24 hours post-mortem, glycogen reserves in the muscle are reduced, lactic acid builds up, and muscle metabolism stops (Lundberg and Vogel 1986). Lactate is a pH lowering by-product of muscle metabolism. Greater amounts of glycogen in the tissue at harvest provide the potential for sustained glycolysis in the muscle after slaughter, which could result in lower ultimate pH. Glycolytic potential is a measure of the amount of energy stored in the muscle at harvest. Huff-Lonergan et al. (2001) showed that glycolytic potential had a significant positive correlation with Hunter L values (0.30) and drip loss (0.36) in our F2 population, and was significantly negatively correlated with color (-0.30) and pH (-0.38). Lower glycolytic potential was associated with a more tender product, with a correlation of –0.31 (Huff-Lonergan et al. 2001).

Very high glycolytic potential values and significantly lower ultimate pH values have been observed in meat from pigs with the Rendement Napole (RN) or acid meat gene (Monin and Sellier, 1985; Milan et al. 2000), which has major effects on meat quality. Based on segregation analysis of phenotypic data, LeRoy et al (1990) first described the RN allele as being dominant. The unfavorable allele reduced water holding capacity, yield of cured cooked ham, and pH, and resulted in lighter colored meat. The RN^- defect has been identified

only in Hampshire pigs and is associated with a 70% increase in muscle glycogen content (LeRoy et al.1990). Recently, Milan et al. (2000) discovered the causative mutation of the RN- effect in the PRKAG3 gene on SSC15 (Protein kinase AMP activated - γ 3 subunit).

In our study, in total 6 QTL were found for glycogen and lactate content and for glycolytic potential, on chromosomes 11, 15, and 17 (Tables 2 and 3), of which 1, on chromosome 15 for glycogen content, was significant at the 5% genome-wise level. On SSC 11, QTL were found for both glycogen content and glycolytic potential at the proximal end of the chromosome. These QTL were in the same region as a QTL for drip loss and likely represent the same locus. Yorkshire alleles were favored for these QTL, with lower glycogen and glycolytic potential and less drip loss. These QTL were not found in other studies and could be specific to these breeds.

Two QTL were detected at the same position on SSC 15, one for glycogen content, which was significant at the 5% genome-wise level, and one for glycolytic potential (Table 2, Fig. 1F). Several additional QTL for other meat quality traits were found to be located in the same region of the chromosome (Table 2, Figs. 1G and H). Further analysis is needed to determine whether these represent the same QTL. Berkshire alleles were superior to Yorkshire alleles for all QTL on SSC 15 (Table 3). Berkshire alleles had lower glycogen content, lower glycolytic potential, lower reflectance, higher pH, better tenderness, and better flavor. These results are consistent with trait correlations (Huff-Lonergan et al. 2001).

Milan et al. (2000) mapped the PRKAG3 (RN) gene between SW1683 and SW1983 (Figs. 1F, G and H), which is central to the QTL regions detected in our study. Further testing revealed, however, that the RN^- mutation found by Milan et al. (2000) is not present

in our population. Additional mutations in this or closely linked genes may be present in our population.

The final QTL for traits associated with glycogen metabolism were detected on SSC 17, with suggestive QTL for lactate content and glycolytic potential (Table 2, Fig. 1I). Both QTL were in the same region as the 5% genome-wise significant QTL for color and reflectance and likely represent the same QTL. Berkshire alleles were favored for the QTL in this region, with lower lactate content and glycolytic potential, and better color and lower reflectance. These QTL were not confirmed by other studies.

Cooking and sensory evaluation traits.

Traits associated with sensory evaluation were measured both objectively, using star probe force as a measure of tenderness, and subjectively, using trained panelists. Huff-Lonergan et al. (2001) showed that lower average star probe force values were associated with better subjective tenderness scores (correlation –0.54) and tenderness of the product, whether measured objectively or subjectively, was moderately correlated with light reflectance in our F2 population. Product that was darker in color (lower Hunter L value) was evaluated as being more tender (lower Star Probe values and higher sensory tenderness scores). Measures of tenderness were also moderately but favorably correlated with drip loss, cooking loss, and subjective evaluations of firmness and juiciness.

A total of nine suggestive QTL were detected for objective and subjective traits associated with tenderness, juiciness and cooking loss. Chromosome 2 showed QTL for two subjective traits related to tenderness (Table 2, Fig. 1B), of which the QTL for tenderness approached 5% genome-wise significance at the distal part of the chromosome. A QTL for

chewiness was detected in the same region on SSC2 as for firmness. Yorkshire alleles were associated with greater tenderness and less chewiness for these QTL.

Suggestive QTL for individual traits associated with tenderness were identified on chromosomes 10 (star probe force), 12 (chewiness), 14 (cooking loss and tenderness), and 17 (juiciness) (Table 2). The QTL for chewiness on SSC 12 was in the same region as the 5% genome-wise significant QTL that was detected for color score.

Suggestive QTL for star probe force and subjective tenderness were detected at the same position on SSC 15 (Table 2). These QTL were in the central region of the chromosome, where also QTL associated with pH and glycogen metabolism were found. Berkshire alleles were associated with greater tenderness.

Except for the QTL on SSC 2, none of our QTL associated with tenderness could be confirmed based on literature results. Andersson-Eklund et al. (1998) found that the proportion of Wild Boar alleles on SSC 3 was associated with shear force but we were not able to confirm this result.

Flavor is an important parameter for meat quality from a consumer perspective. Any flavor that can not be associated with normal pork flavor is considered off-flavor. Flavor and off-flavor scores had a substantial negative correlation in our data (-0.62, Huff-Lonergan et al. 2001). Better flavor scores tended to be associated with higher pH (correlations of 0.25 to 0.32), less glycolytic potention (-0.24) and greater lipid concentration (0.23) (Huff-Lonergan et al. 2001). Opposite relationships held for off-flavor score.

We found three suggestive QTL for off-flavor score and two suggestive QTL for flavor score (Tables 2 and 3). Past studies did not report QTL for these two traits. Chromosome 2 showed QTL for both flavor and off-flavor score but at different positions.

The QTL for flavor score was in the same region as the QTL associated with tenderness, color and reflectance. The QTL for off-flavor score did not appear to be associated with QTL for other traits.

An additional QTL for flavor was detected on chromosome 15 (Table 2). This QTL was in the same region as the QTL for reflectance, pH, and glycogen metabolism that were detected on this chromosome and near the RN gene. Berkshire alleles were associated with more flavor, consistent with the favorable Berkshire effects observed for other traits on this chromosome.

A QTL for off-flavor score was also observed on the X chromosome, which was the only QTL observed across all traits on this chromosome (Table 2). Berkshire alleles were associated with less off-flavor.

Conclusions

Despite the limited differences between the two breeds used in this cross compared to exotic crosses, a total of 60 QTL were detected at the 5% chromosome-wise significance level for the 28 traits evaluated in this study. Of the 60 suggestive QTL, 9, and 1 QTL were significant at the 5%, and 1% genome-wise levels (Table 3), respectively. If no QTL were present for any trait, 28, 1.4, and 0.3 QTL would be detected at these levels by chance alone. We chose to report all QTL significant at the 5% chromosome-wise level. This will aid other researchers as additional experiments are reported for these meat, muscle and sensory traits. Our study reports many QTL that had not been previously reported and we were able to confirm only a limited number of QTL that were described previously. These differences

with literature results may be due to the fact that two commercial breeds were used in our study, compared to literature results, which generally involved one exotic breed.

Significant QTL existed for nearly all traits. They varied in size but most accounted for 3 to 5% of the total F2 variance. Some QTL exceeded this considerably and one QTL reached 10%. Both breeds had favorable QTL on separate chromosomes for meat quality. Overall, chromosomes 15 and 17 contributed highly to the Berkshire superiority in meat quality but Yorkshires were superior for chromosomes 2, 5, and 11. There was some evidence on several chromosomes that cryptic alleles existed which favored the breed least expected to have them.

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Table 1. Means and standard deviations for traits of interest measured on 525 F2 animals and expected differences between breed means (Berkshire minus Yorkshire)[a].

Trait (score range)	Score interpretation		N	Mean	Std Dev	Berk–York[a]
	Low value	High value				
Subjective carcass evaluations						
Color score (1 – 5)	Pale	Dark	525	3.25	0.48	0.2
Marbling (1 – 5)	Low	High	525	3.80	0.73	0.6
Firmness (1 – 5)	Soft	Firm	525	3.42	0.63	0.4
Light Reflectance						
Hormel Ham Minolta (24-hour Semimembranosus Minolta L values)	Dark	Pale	525	17.47	2.90	NA[b]
Hormel Ham Hunter (24-hour Semimembranosus Hunter L values)	Dark	Pale	525	41.65	3.46	NA
Hormel Loin Minolta (24 hour loin Minolta L values)	Dark	Pale	525	21.09	5.20	-0.8
Hormel Loin Hunter (24 hour Hunter L values)	Dark	Pale	525	44.07	6.12	-0.8
Lab Loin Minolta (48 hour loin Minolta L values)	Dark	Pale	525	22.07	3.24	0.0
Lab Loin Hunter (48 hour Hunter L values)	Dark	Pale	525	46.87	3.39	-0.6

[a]Expected difference between breed means based on twice the difference observed in crossbreds in the NPPC genetic evaluation program (Goodwin and Burroughs 1995).
[b]NA: Not available.

Table 1. (continued)

Trait (score range)	Score interpretation		N	Mean	Std Dev	Berk–York[a]
	Low value	High value				
Muscle pH						
Hormel Ham pH (24 hour)	Pale	Dark	525	5.89	0.22	NA
Hormel Loin pH (24 hour)	Pale	Dark	525	5.78	0.17	NA
Lab Loin pH (48 hour)	Pale	Dark	525	5.83	0.19	0.14
Tissue Quality and Water Holding Capacity						
Drip Loss (%)	Low loss	High loss	525	5.84	1.99	-0.84
Water Holding Capacity (g)	Low loss	High loss	525	0.21	0.137	-0.014
Fiber Type I %			513	0.08	0.131	NA
Fiber Type II Ratio			513	1.04	0.77	NA
Glycogen Content of The Loin						
Average Glycogen (μmol/g)			519	8.68	3.34	NA
Average Lactate (μmol/g)			519	86.67	13.30	NA
Average Glycolytic Potential (μmol/g)			518	104.00	16.31	NA

Table 1. (continued)

Trait (score range)	Score interpretation Low value	High value	N	Mean	Std Dev	Berk–York[a]
Fat Content						
Total Lipid (%)			525	3.23	1.32	0.16
Cholesterol (mg/100g)			525	57.72	8.29	0.6
Instrumental Tenderness						
Average Instron (Star Probe) Force (kg)	Tender	Tough	513	7.84	1.17	0.48
Cooking and Sensory Panel Evaluation						
Percent Cooking Loss (%)			513	18.23	4.40	-2.0
Tenderness Score (1-10)	Tough	Tender	488	4.36	0.86	-0.78
Juiciness Score (1-10)	Dry	Juicy	513	6.02	1.49	0.0
Chewiness Score (1-10)	Soft	Tough	513	2.42	0.93	-0.32
Flavor score (1-10)	Little flavor	Intense flavor	513	2.85	1.76	0.0
Off Flavor Score (1-10)	No off flavor	High off flavor	513	1.59	2.03	0.0

Table 2. Evidence for QTL significant at the 5% chromosome-wise level for various meat quality traits by chromosome. Estimated significance levels (F value), location, gene effects and % of F2 variance explained by each QTL.

SSC	Trait	F-value[a]	Location	Additive		Dominance		% variancec
			(cM)	effect[b]	S.E.	effect	S.E.	
1	Marbling	8.42*	48	-0.16	0.04	0.16	0.07	4.34
1	Total Lipid (%)	6.06	51	-0.28	0.08	0.13	0.13	2.90
1	Drip Loss (%)	7.15	90	-0.53	0.14	-0.13	0.27	4.66
2	Color Score	5.33	141	-0.10	0.03	0.04	0.05	2.41
2	Hormel Loin Minolta	5.90	77	0.91	0.31	-0.94	0.48	3.83
2	Lab Loin Minolta	7.24	127	0.83	0.22	0.19	0.38	3.94
2	Lab Loin Hunter	6.33	128	0.80	0.23	0.18	0.39	3.39
2	Drip Loss (%)	5.68	122	0.43	0.13	-0.26	0.23	3.53
2	Drip Loss (%)	5.07	40	0.44	0.14	0.12	0.27	3.22
2	Water Holding Capacity (g)	5.85	139	0.03	0.01	-0.01	0.01	2.67
2	Water Holding Capacity (g)	5.90	71	0.03	0.01	0.01	0.01	2.94
2	Chewiness Score	6.76	143	0.20	0.06	0.07	0.08	2.62
2	Tenderness Score	7.99	143	-0.26	0.07	-0.15	0.11	3.08
2	Firmness	5.31	86	-0.11	0.04	-0.09	0.06	2.39
2	Flavor score	5.93	143	-0.35	0.10	0.03	0.15	2.45
2	Off Flavor Score	5.84	45	0.50	0.15	0.03	0.30	4.18
2	Off Flavor Score	5.17	143	0.35	0.11	0.07	0.16	2.08
4	Lab Loin Hunter	6.15	130	0.54	0.20	0.67	0.29	2.66
4	Lab Loin Minolta	6.01	130	0.55	0.19	0.56	0.28	2.58
5	Hormel Loin Minolta	6.88	112	0.64	0.30	-1.38	0.46	4.11
5	Hormel Loin pH	8.56*	113	-0.03	0.01	0.05	0.02	4.85
5	Lab Loin Hunter	7.41	113	0.48	0.22	-1.09	0.34	4.30
5	Lab Loin Minolta	7.95	113	0.49	0.21	-1.07	0.33	4.59
5	Lab Loin pH	6.20	81	-0.04	0.01	0.01	0.17	3.38
6	Hormel Ham pH	6.82	53	-0.03	0.01	0.05	0.02	2.90
7	Lab Loin Hunter	5.83	80	0.52	0.21	-0.79	0.33	3.00
8	Fiber Type I	5.97	52	-0.03	0.01	-0.01	0.01	2.88
8	Marbling	5.92	40	-0.14	0.05	0.16	0.08	3.61

[a]Chromosome-wise F-statistic thresholds at the 5% level, as determined by permutation test were as follows: (1) 5.08, (2) 5.12, (3) 5.14, (4) 5.14, (5) 4.99, (6) 5.32, (7) 5.25, (8) 5.03, (9) 5.09, (10) 5.11, (11) 4.59, (12) 4.78, (13) 5.03, (14) 5.02, (15) 5.02, (16) 4.34, (17) 4.86, (18) 4.45, (X) 4.80.

[b]Additive (a) and dominance (d) QTL effects correspond to genotype values of +a, d, and –a for, respectively, individuals having inherited two Berkshire alleles, heterozygotes, and individuals with two Yorkshire alleles. Positive additive effects indicate that Berkshire alleles increased the trait, negative that the Berkshire alleles decreased it. Dominance effects are relative to the mean of the two homozygotes.

[c] % variance = genetic variance at the QTL based on estimated additive and dominance effects and allele frequencies of ½, as a percent of the residual variance in the F2.

* Significant at the 5% genome-wise level (F>8.22)

** Significant at the 1% genome-wise level (F> 9.96)

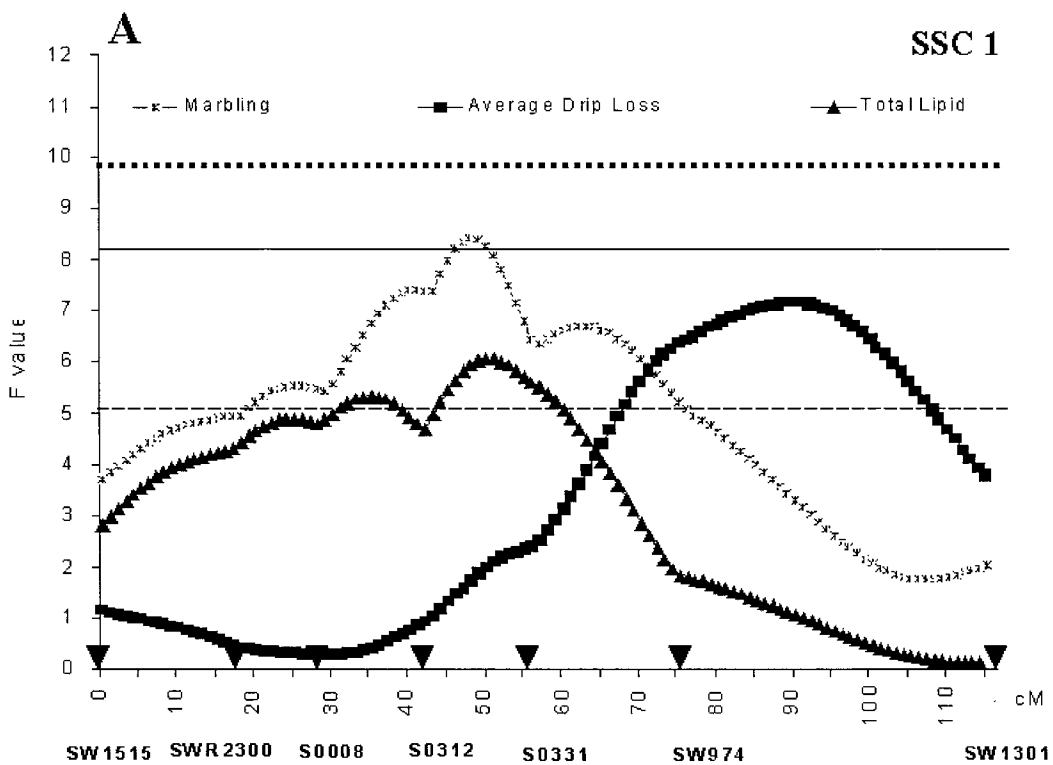
Table 2. (continued)

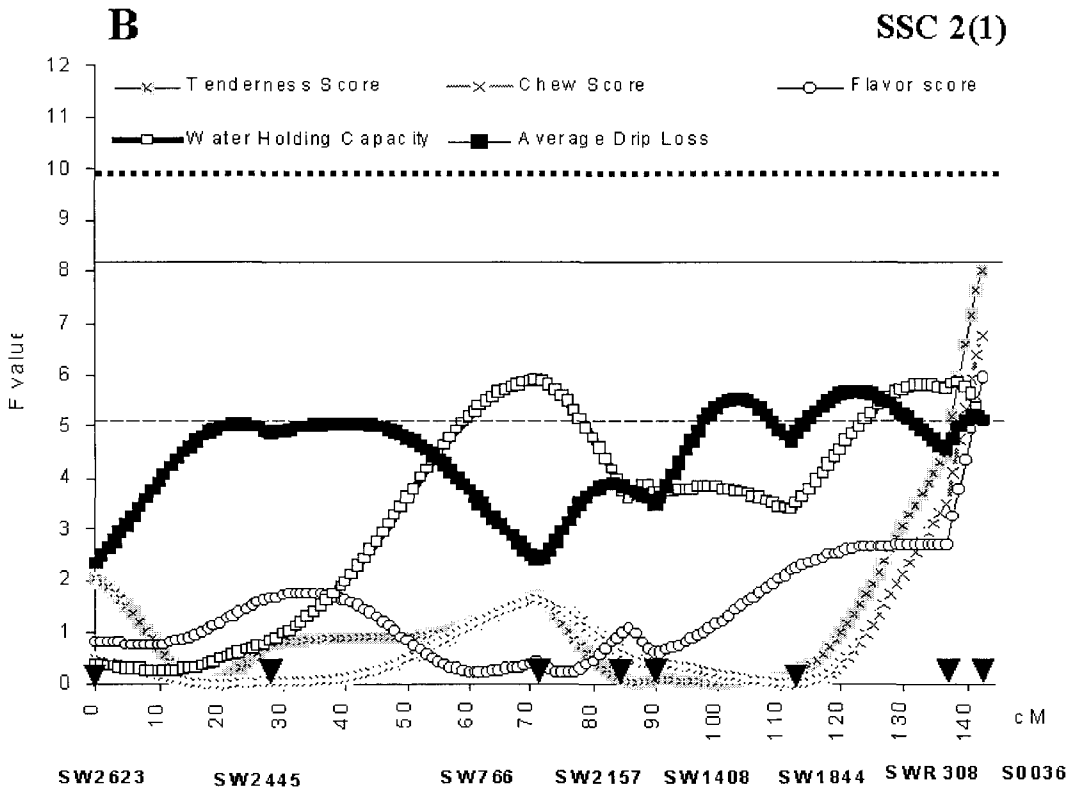
SSC	Trait	F-value[a]	Location (cM)	Additive effect[b]	Additive S.E.	Dominance effect	Dominance S.E.	% variance[c]
10	Star Probe Force (kg)	5.83	71	-0.20	0.06	-0.06	0.10	3.82
10	Marbling	5.11	3	-0.14	0.05	-0.13	0.10	3.24
11	Drip Loss (%)	5.95	7	0.44	0.13	0.59	0.23	6.01
11	Glycogen (*u*mol/g)	4.73	0	0.65	0.21	-0.12	0.34	2.33
11	Glycolytic Potential (*u*mol/g)	5.91	0	3.36	1.03	-1.54	1.66	2.68
12	Chewiness Score	5.13	73	0.10	0.08	0.43	0.14	6.63
12	Color Score	8.33*	73	-0.14	0.04	-0.22	0.07	10.13
13	Water Holding Capacity (g)	6.14	43	0.03	0.01	0.09	0.01	2.75
14	Hormel Ham Hunter	5.16	0	-0.21	0.22	-1.06	0.35	3.19
14	Hormel Ham pH	5.79	110	-0.05	0.02	0.01	0.03	3.59
14	Percent Cooking Loss (%)	7.14	31	-1.03	0.28	-0.40	0.47	3.29
14	Tenderness Score	5.77	70	0.28	0.08	0.23	0.11	2.83
15	Hormel Loin Hunter	6.31	96	-1.07	0.32	0.62	0.50	3.16
15	Lab Loin Hunter	5.04	66	-0.68	0.22	0.17	0.33	2.46
15	Lab Loin Minolta	6.30	66	-0.73	0.21	0.17	0.31	3.05
15	Hormel Ham pH	8.42*	72	0.05	0.01	-0.02	0.02	4.00
15	Hormel Loin pH	12.15**	76	0.05	0.01	-0.01	0.02	5.61
15	Lab Loin pH	9.05*	45	0.04	0.01	-0.04	0.02	5.14
15	Glycogen (*u*mol/g)	8.25*	65	-0.77	0.22	0.71	0.34	4.27
15	Glycolytic Potential (*u*mol/g)	6.21	67	-3.67	1.05	0.77	1.59	2.95
15	Tenderness Score	5.22	44	0.24	0.08	-0.20	0.14	3.00
15	Star Probe Force (kg)	5.25	42	-0.17	0.05	0.09	0.09	2.88
15	Flavor score	6.41	91	0.36	0.11	-0.37	0.18	3.73
17	Color score	8.75*	82	0.11	0.03	-0.09	0.04	3.63
17	Lab Loin Hunter	9.11*	82	-0.83	0.20	0.22	0.29	3.73
17	Lab Loin Minolta	9.91*	82	-0.83	0.19	0.25	0.28	4.04
17	Lactate (*u*mol/g)	6.40	82	-1.48	0.77	3.37	1.11	2.80
17	Glycolytic Potential (*u*mol/g)	5.01	82	-1.47	0.10	4.05	1.44	2.22
17	Juiciness Score	6.36	30	0.23	0.12	-0.70	0.24	8.03
18	Hormel Loin Minolta	6.40	26	0.12	0.29	-1.58	0.45	3.82
18	Cholesterol (mg/100g)	4.67	26	-0.15	0.56	2.60	0.86	2.62
X	Off Flavor Score	4.90	69	-0.58	0.19	-0.12	0.20	5.78

Table 3. Summary of QTL significant at the 5% chromosome-wise level (%5 chr), the 5% genome-wise level (%5 gen) (F>8.22) and the 1% genome-wise level (%1 gen) (F>9.96) by trait.

Trait	# of significant QTL			% of F_2 variance explained
	%5 chr	%5 gen	%1 gen	
Marbling	2	1		11.2
Total Lipid %	1			2.9
Cholesterol Concentration	1			2.6
Hormel Ham pH	2	1		10.5
Hormel Loin pH		1	1	10.5
Lab Loin pH	1	1		8.5
Hormel Loin Hunter Score	1			3.2
Hormel Ham Hunter Score	1			3.2
Lab Loin Hunter Score	5	1		19.5
Hormel Loin Minolta	3			11.8
Lab Loin Minolta	4	1		18.2
Color Score	1	2		16.2
Firmness	1			2.4
Water Holding Capacity	3			8.4
Drip Loss	4			17.4
Percent Cooking Loss	1			3.3
Juiciness Score	1			8.0
Star Probe Force	2			6.7
Tenderness Score	3			8.9
Chewiness Score	2			9.3
Flavor Score	2			6.2
Off Flavor Score	3			12.0
Lactate	1			2.8
Glycogen	1	1		6.6
Glycolytic Potential	3			7.9
Fiber Type I	1			2.9

Figure 1. F-ratio curves for evidence of QTL. The x-axis indicates the relative position on the linkage map. The y-axis represents the F-ratio. Arrows on the x-axis indicate the position where a marker was present. Three lines are provided for 5% chromosome-wise (------), 5% genome-wise (_____) and the 1% genome-wise (.....) significance.




B
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Tenderness Score
Chew Score
Flavor score
Water Holding Capacity
Average Drip Loss
F value
cM
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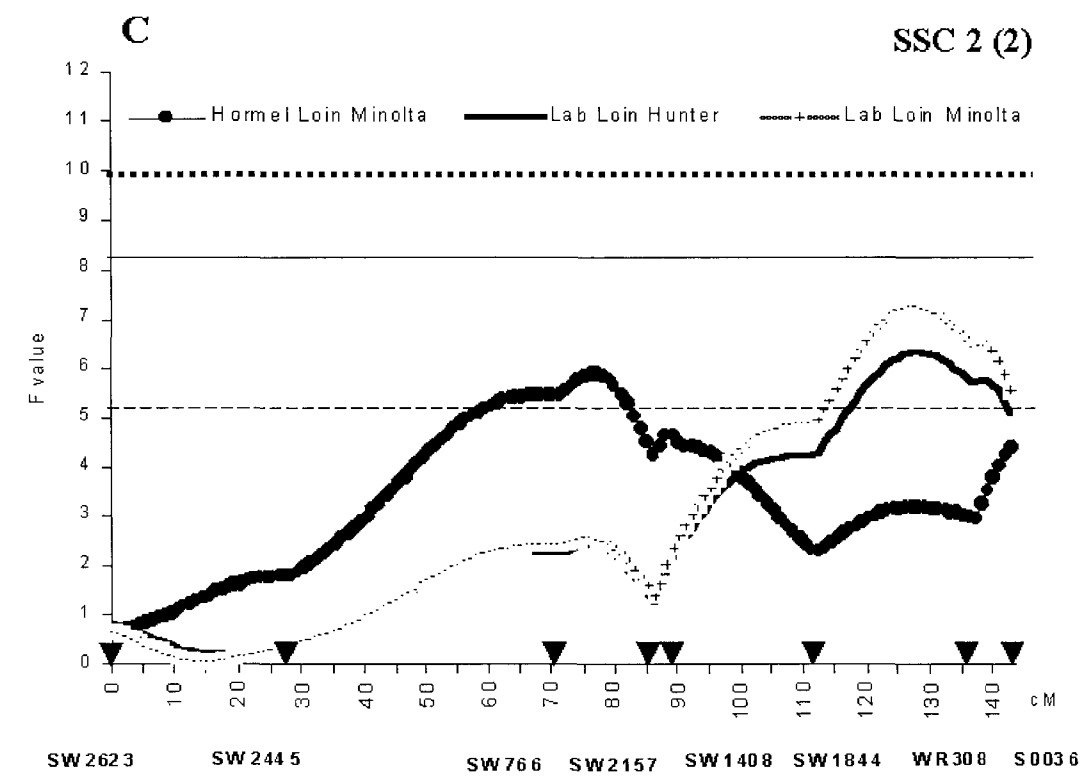
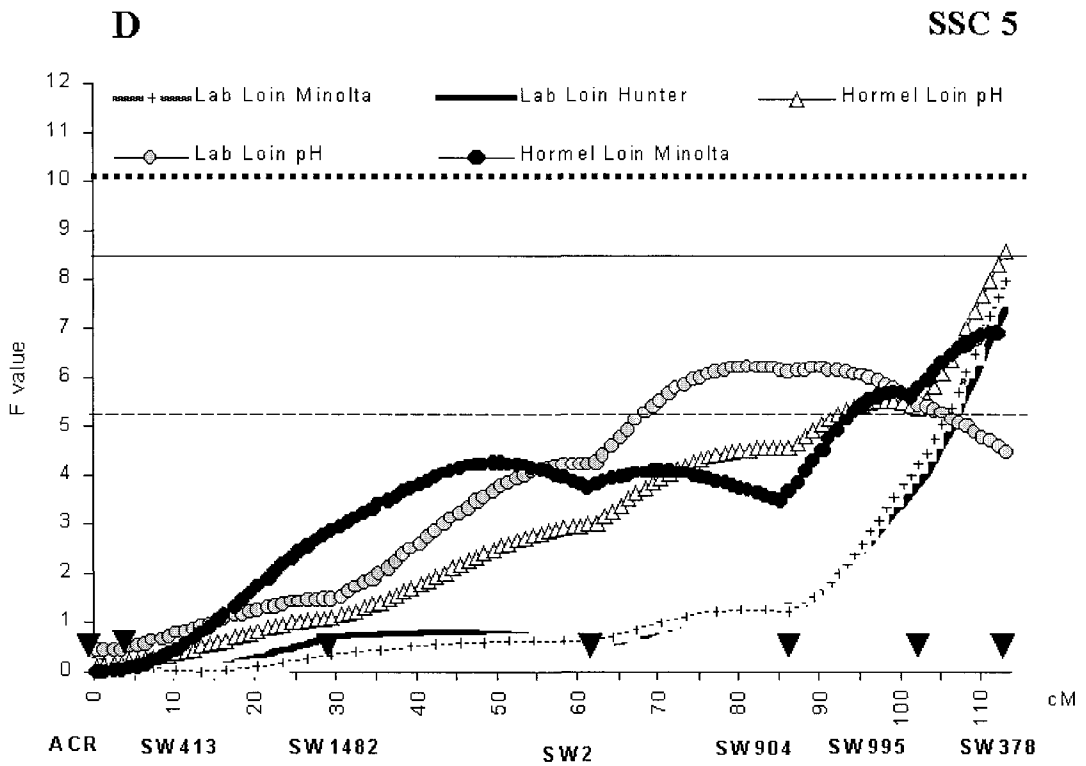
SW2445
SW766
SW2157
SW1408
SW1844
SWR308
S0036


C
SSC 2 (2)
Hormel Loin Minolta
Lab Loin Hunter
Lab Loin Minolta
F value
cM
SW2623
SW2445
SW766
SW2157
SW1408
SW1844
WR308
S0036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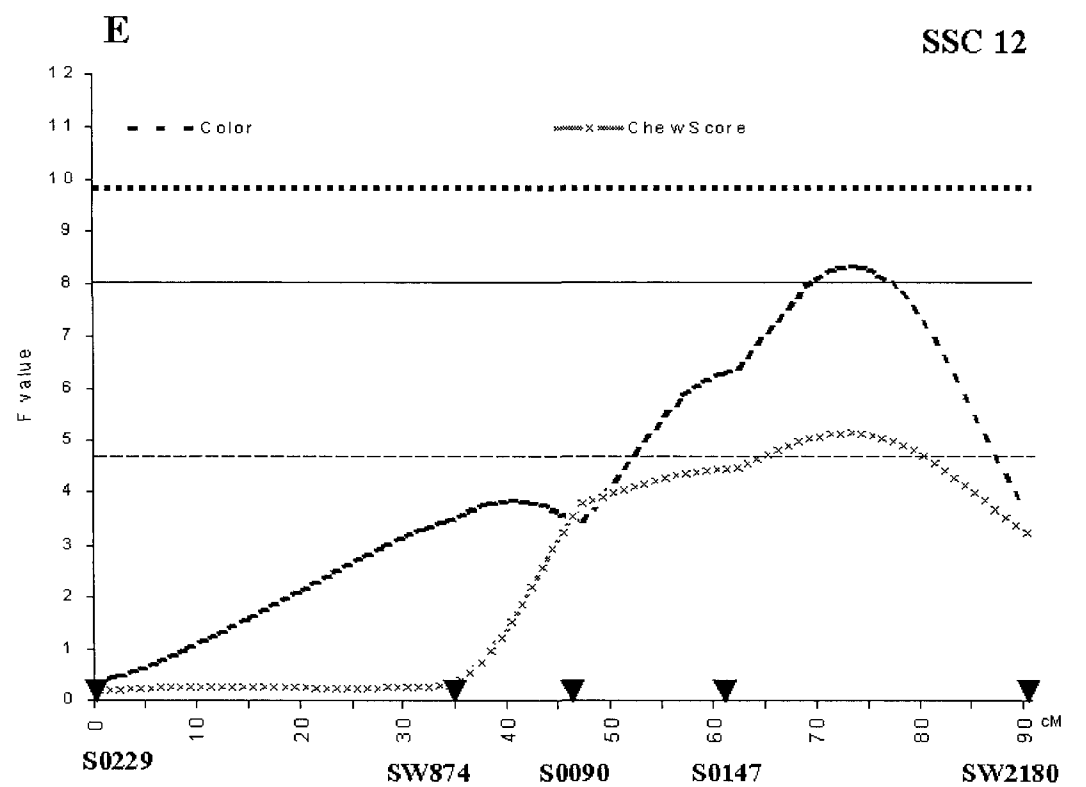
SSC 5
Lab Loin Minolta
Lab Loin Hunter
Hormel Loin pH
Lab Loin pH
Hormel Loin Minolta
F value
cM
ACR
SW413
SW1482
SW2
SW904
SW995
SW378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Color
Chew Score
F value
cM
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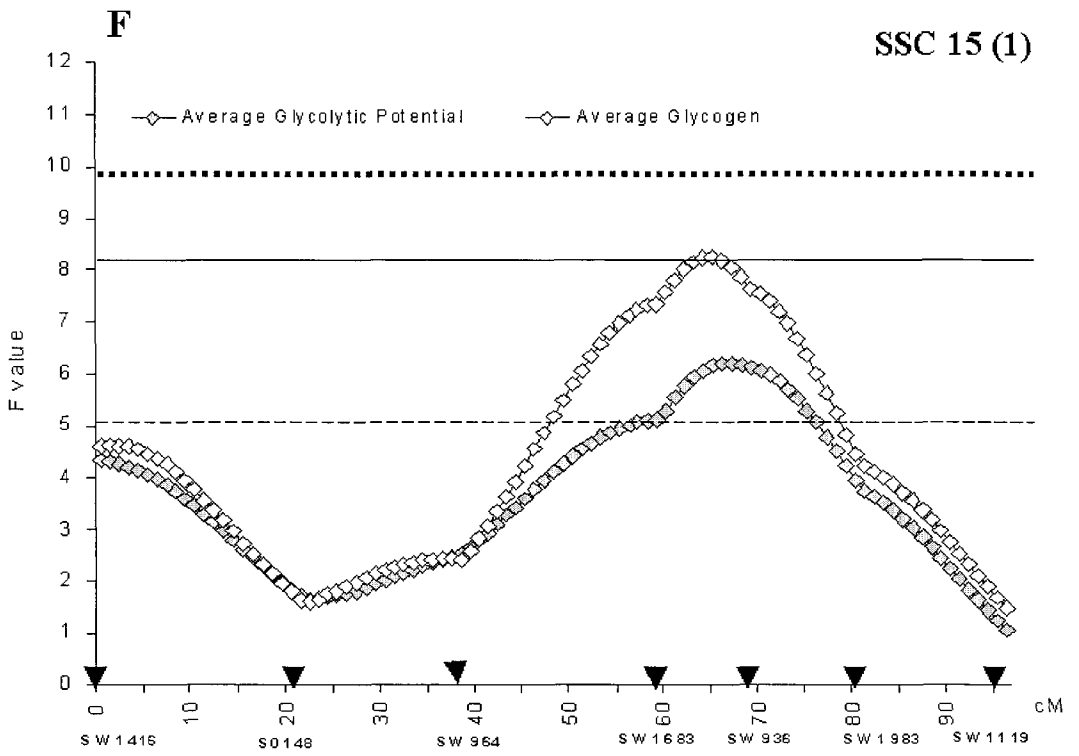
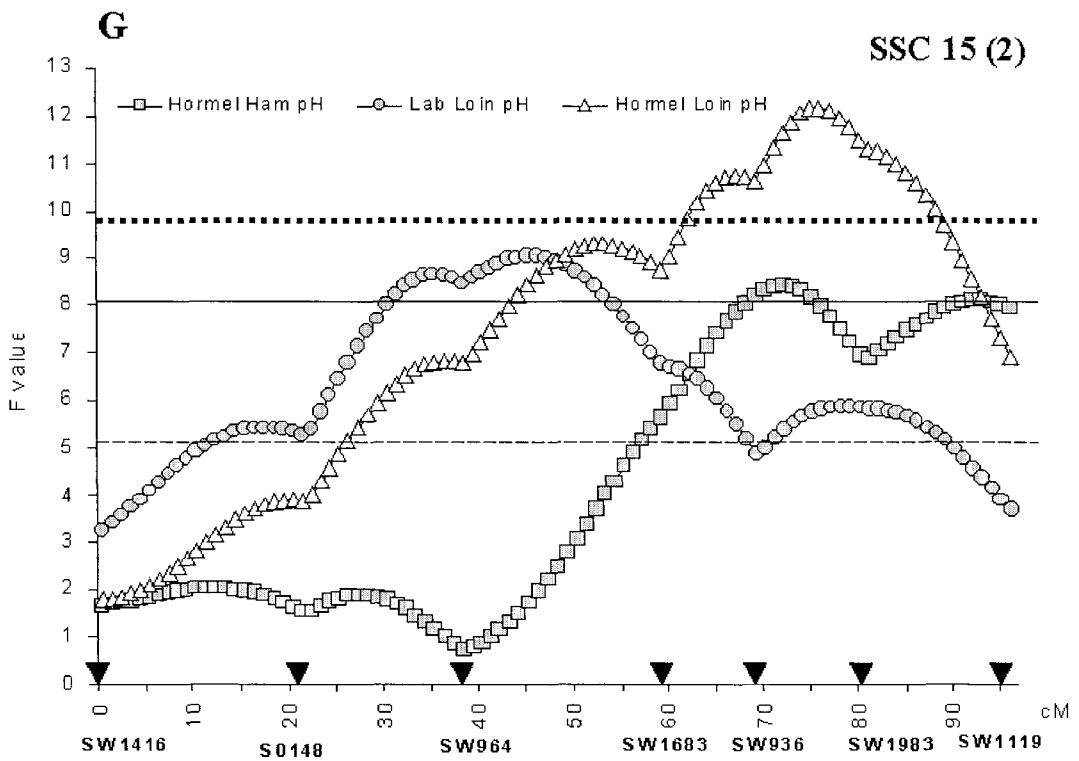
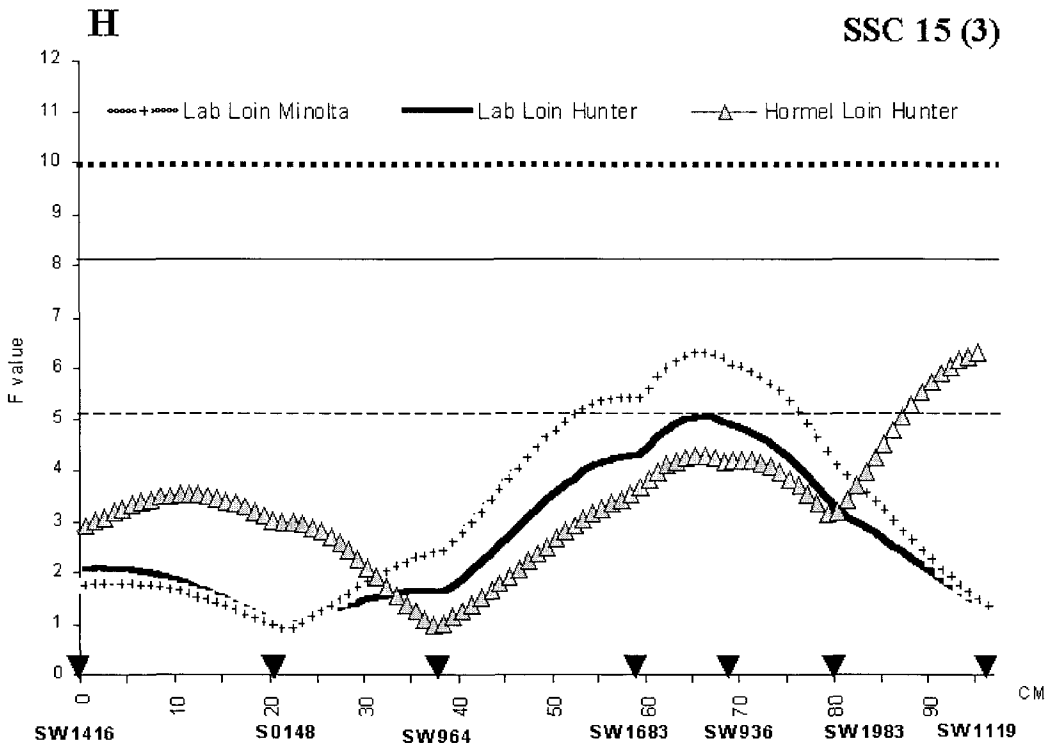
S0090
S0147
SW2180


F
SSC 15 (1)
Average Glycolytic Potential
Average Glycogen
F value
cM
SW1415
S0148
SW964
SW1683
SW936
SW1983
SW1119


G
SSC 15 (2)
Hormel Ham pH
Lab Loin pH
Hormel Loin pH
F value
cM
SW1416
S0148
SW964
SW1683
SW936
SW1983
SW1119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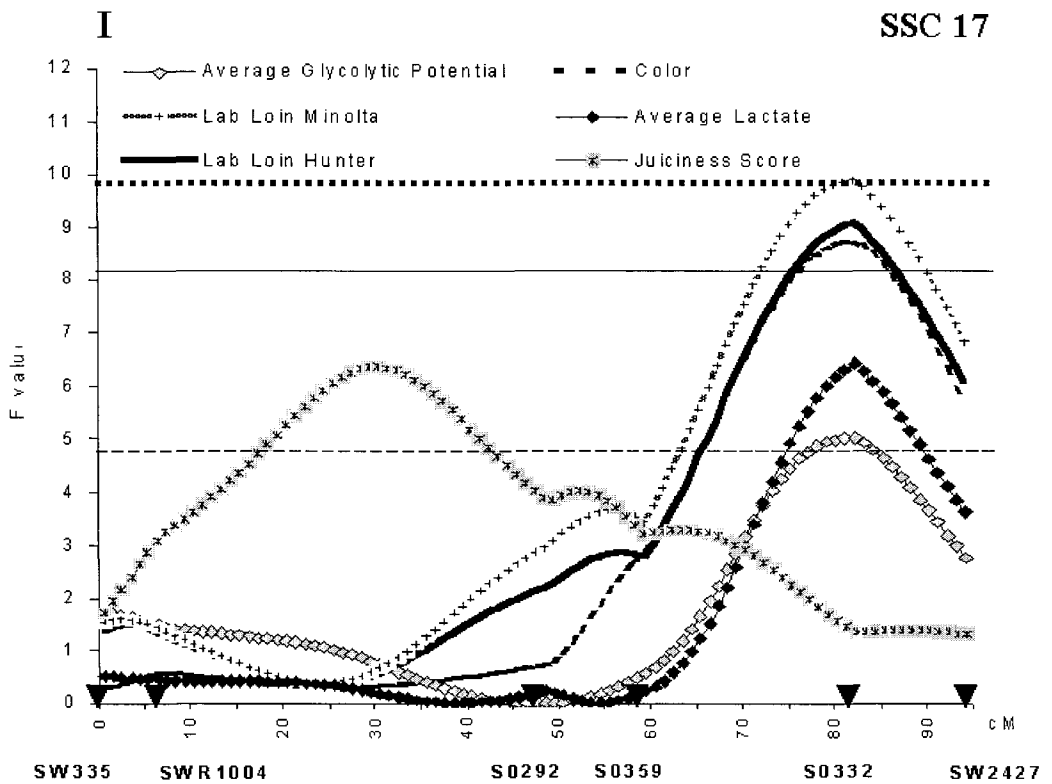
SSC 15 (3)
Lab Loin Minolta
Lab Loin Hunter
Hormel Loin Hunter
F value
CM
SW1416
S0148
SW964
SW1683
SW936
SW1983
SW1119


I
SSC 17
Average Glycolytic Potential
Color
Lab Loin Minolta
Average Lactate
Lab Loin Hunter
Juiciness Score
CM
SW335
SWR1004
S0292
S0359
S0332
SW2427

CHAPTER 4. GENETIC AND PHYSICAL MAPPING OF *ACACB, PPP1CC* AND *GPR49* GENES ON PORCINE CHROMOSOMES 5 AND 14 HELP TO DEFINE A BREAK POINT ON HUMAN CHROMOSOME 12.

A paper to be submitted to Animal Genetics

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Summary

The mapping of genes found together on a single human chromosome can help to define breakpoints relative to the porcine genome map. Several primer sets were designed based on human DNA sequences to amplify: protein phosphatase 1 catalytic subunit, gamma (*PPP1CC*), acetyl-CoA carboxylase, beta (*ACACB*), and G protein-coupled receptor 49 (*GPR49*). Using a somatic cell hybrid panel, the *GPR49* and *PPP1CC* genes were physically mapped. The *GPR49* gene mapped to pig chromosome 5p11-15 and *PPP1CC* mapped to pig chromosome 14. Linkage mapping of the *ACACB* and *GPR49* genes was performed using identified single nucleotide polymorphisms (SNPs) and a three generation Berkshire x Yorkshire (B x Y) pig resource family. The porcine *GPR49* gene was mapped to chromosome 5 (SSC5) with the most likely position being between SW1482 and SW2, confirming the physical localization. The porcine *ACACB* gene mapped to chromosome 14 (SSC14), with the *ACACB* gene being located between SW1027 and SWC6. No SNP was found for *PPP1CC* to confirm its physical mapping on chromosome 14. The results presented here are valuable for establishing the position of three genes located closely

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together on human chromosome 12, but present on two different pig chromosomes (5 and 14) due to the presence of a chromosome breakpoint.

Key Words: Porcine, *PPP1CC*, *ACACB*, *GPR49*, Polymorphism, Gene Mapping.

Meat quality contributes considerably to the profit involved in pork production. Producers that have pigs with better pork quality will be paid a higher market price. In recent years there has been a significant effort to improve pork quality. Genomic solutions are being considered to speed up this process. One of the main ideas behind genomic research in farm animals has been the mapping and identification of genes underlying economically important traits like meat quality (Andersson 2001). Due to the rapid development of molecular biology during the past decade, several genes with major effects have been found to affect body composition and meat quality, including the Halothane (*HAL*) or stress gene (Christian 1972; Fujii et al. 1991), the Rendement Napole (*RN*) or acid meat gene (Monin & Sellier 1985; LeRoy et al. 1990; Milan et al. 2000), and *H-FABP* and *A-FABP* (Gerbens et al. 1998). Comparative mapping is one of the approaches used to fine map chromosomal regions and improve our ability to find genes responsible for traits of interests. Currently, a need exists to improve the pig gene map by mapping specific genes that have been located on the human map. This will allow animal geneticists to take advantage of the significant resources in the human genome project.

The main objective of this study was to have a better understanding of a breakpoint in human chromosome 12 relative to the pig genome. Narrowing down the location of the breakpoint is important not only in respect to the comparative mapping effort but also opens the possibility of using a positional candidate gene approach to identify genes responsible for

QTL for meat quality and body composition that have been reported on SSC 5 (Malek et al. 2001). This approach will help to increase the number of genes mapped in this region and to investigate possible candidate genes.

The genes mapped in this study were chosen based on their location in the human map and also by their function as possible candidate genes affecting meat quality. Type-1 protein phosphatase (pp1) is essential for cell division and it participates in the regulation of glycogen metabolism, muscle contractility and protein synthesis. Gamma subunit (PPP1CC) is one of the catalytic subunits of the phosphatase. Acetyl-CoA carboxylase, beta (ACACB) may be involved in the provision of malonyl-coA or in the regulation of fatty acid oxidation, having three functions: biotin carboxyl carrier protein, biotin carboxylase, and carboxyltransferase (Saddik et al. 1993). The G protein-coupled receptor 49 (*GPR49*), is a novel member of the glycoprotein hormone receptor subfamily with approximately 35% overall identity at the protein sequence level (McDonald et al. 1998).

The primer sets A, D and G, which were used to amplify all three genes, were designed based on human sequences available in GenBank (Accession No: *GPR49,* AF062006; *PPP1CC,* XM_007017*; ACACB,* XM_017203*)* and are presented in Table 1. The PCR were performed using 12.5 ng of porcine genomic DNA, optimum concentration of $MgCl_2$ (Table1), 0.2 m*M* dNTP, 0.25 μ*M* of each primer, 0.35 U *Taq* DNA polymerase (Promega, Madison, WI) and PCR buffer (10 m*M* Tris-HCl, 50 m*M* KCl and 0.1% Triton®X-100) in a 10μl final volume. The cycling conditions included initial denaturation at 94° C for 3 min followed by 30 cycles at 93° C for 45 s, optimum annealing temperature for 1 min (Table1), 72° C for 1 min, and final extension at 72° C for 10 min. The reverse transcription was performed only in the case of the *PPP1CC* gene using total RNA (3.5μg),

random hexanucleotide priming and Superscript II (GIBCO/BRL, Rockville, MD), according to the manufacturer's protocol. The PCR and RT-PCR products from several individuals from each of the commercial breeds of pigs (Landrace, Large White, Duroc, Hampshire and Berkshire) were directly sequenced using dye terminators and an ABI 377 sequencer (Perkin Elmer, Foster City, CA).

Physical mapping of the porcine *GPR49* and *PPP1CC* genes was performed using a pig/rodent somatic cell hybrid panel (SCHP) of 27 cell lines (Yerle et al. 1996), using B and F pig specific primers sets respectively. All the PCR products were separated by agarose electrophoresis and visualized by ethidium bromide staining. The PCR results were submitted and analyzed as described on the Web site http://www.toulouse.inra.fr/lgc/pig/hybrid.htm.

The results of the SCHP analysis revealed that *GPR49* is located on pig chromosome 5p11-15 (Figure 1). The *PPP1CC* gene was mapped to pig chromosome 14 region D_1 (not integrated yet). Based on bi-directional chromosome painting the region D_1 is more likely to be present on the 14q21-22 region (Goureau et al. 1996; http://www.toulouse.inra.fr/lgc/pig/compare/SSCHTML/SSC3B.HTM). In humans, *PPP1CC* was mapped to 12q24.1-24.2 and *GPR49* to 12q22-23 (http://bioinformatics.weizmann.ac.il/cards/).

Sequencing revealed single nucleotide polymorphisms (SNPs) in the 5' UTR and exon 18 regions of *ACACB* and *GPR49,* respectively. Primer set "I" was used to amplify a fragment of *ACACB* and a restriction site for *Hpa*I was introduced using a modified forward primer and confirmed by enzyme digestion by PCR-RFLP. This polymorphism was an A to G. The fragments sizes generated by the *Hpa*I digestion were 235bp (allele 1) and 200bp and 35bp (allele 2). A PCR product was generated for *GPR49* using the "C" primer set and a

*Pvu*II restriction site was introduced using a modified reverse primer. The SNP was confirmed by enzyme digestion using a PCR-RFLP and the polymorphism was a C to T substitution. The fragments sizes generated by the *Pvu*II digestion were 458bp (allele 1) and 424bp and 34bp (allele 2). For linkage mapping, the B x Y resource family was genotyped (Malek et al. 2001) for the *Pvu*II *GPR49* and *Hpa*I *ACACB* polymorphisms and two-point and multipoint linkage analyses were performed using the CRI-MAP program (Green et al. 1990). The segregation of the *Hpa*I and *Pvu*II polymorphisms followed a Mendelian autosomal inheritance pattern. No SNP was discovered in the *PPP1CC* gene.

The results of the linkage analysis showed that *GPR49* gene was linked to four markers on SSC5 (the two point recombination frequencies and LOD scores with values greater than 6.00 are given in parentheses): SW1482 (0.30, 7.11), SW2 (0.06, 33.07), SW904 (0.26, 11.92) and *IGF1* (0.32, 6.37). These results and multipoint linkage analysis show that the *GPR49* gene is most likely located between SW1482 and SW2, confirming the physical mapping localization. Linkage mapping analysis was used also to map the *ACACB* gene on SSC14. The results of two-point analysis showed that the *ACACB* gene was linked to several markers on SSC14: SW1027 (0.24, 18.92), SWC6 (0.11, 51.16), SWR84 (0.20, 26.32), S0007 (0.26, 15.70) and SW77 (0.29, 11.30). The results from multipoint analysis showed that the *ACACB* gene is located between SW1027 and SWC6. In accordance with the results obtained by chromosome painting between human and pig (Goureau et al. 1996), this is gene expected to map near *PPP1CC*. The results of *ACACB* linkage analysis confirmed this information. Based on all this information we consider the break point location is more likely to be in the q23 region (Figure 1).

The results presented here are valuable for establishing the position of three genes that are closely located on human chromosome 12 that now appear to be on two different pig chromosomes (5 and 14) due to the presence of a chromosome breakpoint.

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Table 1. The primer sequences and PCR conditions for each of the studied genes.

Locus Symbol	Primer Set		Primers (5'- 3')[a]	PCR conditions [b]		
				bp	T	{$MgCl_2$}
GPR49	A	F	GAG TGT GGA CCA TAG CAG TT	680	53.3°C	1.5
		R	AGA GGA GAA GGA CAA GAA AG			
GPR49	B	F	CGG AGC GTG GTG GGA GCA	680	62.3°C	1.5
		R	TAG AGC TTG GTG TAG GCG ATT			
GPR49	C	F	GAG TGT GGA CCA TAG CAG TT	458	58°C	1.5
		R	TTTTTTTTTTTTTTCGGGGAGGCGCTGTACCAGCT			
PPP1CC	D	F	GAC CAA CTG ATG TAC CAG ATC	435	55°C	1.5
		R	AAA CAG AGT GAC CAA CTG CCT C			
PPP1CC	E	F	ATG GCG GAT TTA GAT AAA CTC AA	890	55°C	1.5
		R	AA CAG AGT GAC CAA CTG CCT C			
PPP1CC	F	F	GAC CAA CTG ATG TAC CAG ATC	368	55°C	1.5
		R	GTT TTC AGT ATA AGT AGG TGA GA			
ACACB	G	F	TGA TTT TCT CCT GTC TGA CC	510	53.3°C	1.25
		R	AGA TGA GCC AGC AAC AGA GT			
ACACB	H	F	TCC TGT CTG ACC TTT TCC TG	510	53.3°C	1.25
		R	AGA TGA GCC AGC AAC AGA GT			
ACACB	I	F	AAAAAAAAAAAAAATCCCCGCAGCACCCCAAGTTA	235	52°C	1.5
		R	AGA TGA GCC AGC AAC AGA GT			

Table 1. The primer sequences and PCR conditions for each of the studied genes.

Locus Symbol	Primer Set		Primers (5'- 3')[a]	PCR conditions [b]		
				bp	T	{$MgCl_2$}
GPR49	A	F	GAG TGT GGA CCA TAG CAG TT	680	53.3°C	1.5
		R	AGA GGA GAA GGA CAA GAA AG			
GPR49	B	F	CGG AGC GTG GTG GGA GCA	680	62.3°C	1.5
		R	TAG AGC TTG GTG TAG GCG ATT			
GPR49	C	F	GAG TGT GGA CCA TAG CAG TT	458	58°C	1.5
		R	TTTTTTTTTTTTTTCGGGGAGGCGCTGTACCAGCT			
PPP1CC	D	F	GAC CAA CTG ATG TAC CAG ATC	435	55°C	1.5
		R	AAA CAG AGT GAC CAA CTG CCT C			
PPP1CC	E	F	ATG GCG GAT TTA GAT AAA CTC AA	890	55°C	1.5
		R	AA CAG AGT GAC CAA CTG CCT C			
PPP1CC	F	F	GAC CAA CTG ATG TAC CAG ATC	368	55°C	1.5
		R	GTT TTC AGT ATA AGT AGG TGA GA			
ACACB	G	F	TGA TTT TCT CCT GTC TGA CC	510	53.3°C	1.25
		R	AGA TGA GCC AGC AAC AGA GT			
ACACB	H	F	TCC TGT CTG ACC TTT TCC TG	510	53.3°C	1.25
		R	AGA TGA GCC AGC AAC AGA GT			
ACACB	I	F	AAAAAAAAAAAAAATCCCCGCAGCACCCCAAGTTA	235	52°C	1.5
		R	AGA TGA GCC AGC AAC AGA GT			

CHAPTER 5. POSITIONAL CANDIDATE GENE ANALYSIS OF QUANTITATIVE TRAIT LOCI FOR MEAT QUALITY ON PORCINE CHROMOSOME 5.

A paper to be submitted to Mammalian Genome

Massoud Malek[1], Daniel C. Ciobanu, Jack C. M. Dekkers, and Max F. Rothschild

Abstract

Pork is one of the important animal protein sources worldwide. Increased consumer concerns for the quality of pork has caused meat quality traits to become an important factor for pig producers. The objective of this study was to conduct a positional candidate gene analysis of QTL regions on chromosome 5 in the pig, which were detected in a previous genome scan for meat quality and body composition using a three generation Berkshire x Yorkshire (BxY) resource family. Two candidate genes were considered: the ATPase, Ca^{2+} transporting, plasma membrane 1 gene (*ATP2B1)* and the dual specificity phosphatase 6 (*DUSP6*) gene. Single nucleotide polymorphisms were identified and used to map these genes to regions on chromosome 5 with previously identified QTL for fat content and light reflectance. Association analyses on the BxY F_2 population revealed significant effects of *ATP2B1-Afl*III genotypes for carcass length ($p<0.01$), loin eye area ($p<0.01$), tenth rib back fat ($p<0.1$), marbling ($p<0.01$), average daily gain on test ($p<0.05$), average glycogen ($p<0.1$), average glycolytic potential ($p<0.1$), and ham and loin pH ($p<0.1$). Significant effects were also revealed for *DUSP6-Pst*I genotypes for lumbar

($p<0.01$), average back fat ($p<0.1$), 16-day weight ($p<0.01$), average daily gain to weaning ($p<0.004$), and average daily gain on test ($p<0.1$). In order to verify these results, five closed pig commercial lines were examined for associations of *DUSP6-Pst*I and *ATP2B1-Afl*III genotypes with meat quality traits. One line was polymorphic for *ATP2B1-Afl*III and this line showed an association that was significant ($p<0.1$) with 24-hr ham Minolta. The *DUSP6-Pst*I polymorphism was polymorphic in two lines and found to be associated with light reflectance (loin Minolta) and backfat thickness in both lines and with 24-hr loin pH loin depth in one of these lines. The results of haplotype analysis in one line that was polymorphic for both genes confirmed that the *ATP2B1* gene was associated with ham Minolta (a and b) traits. The results of haplotype analysis also showed that the region around *DUSP6* gene is associated with body composition traits. The haplotype results for carcass backfat thickness measured on the carcass were in line with the haplotype results for ultrasound backfat and lean meat percentage of the carcass. Combined, the QTL analysis and the association studies in the BxY and commercial lines confirm that fat traits appear to be associated with the *DUSP6* gene and that variation in the *ATP2B1* gene is associated with variation in meat quality. The use of these genes in marker assisted selection can result in substantial improvements.

Key Words: Porcine, *DUSP6, ATPB1,* Polymorphism, Gene Mapping.

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Introduction

The pork industry has been progressing rapidly to meet a demand for improved meat quality, which is one of today's key consumer concerns. However, genetic improvement of meat quality is difficult by traditional breeding programs because most meat quality traits cannot be measured on live animals and some traits have low heritability. Marker assisted selection has been suggested as a good approach to overcome this problem by using information from QTL (quantitative trait loci) for selection (Meuwissen and Goddard 1996)

Known functional roles of genes and their genomic locations in different species such as humans can be used to find positional candidate genes for trait of interest (Lander et al. 2001). Comparative mapping knowledge has grown rapidly over the past few years. This has allowed researchers to take advantage of the significant resources in the human genome project. Therefore, specific genes identified in the human genome in regions that are syntenic with a specific region in the pig, as identified using bi-directional chromosomal painting (Goureau et al. 1996), could be a source of positional candidate genes in the pig for traits of interests.

Due to the rapid development of molecular biology during the past two decades, several genes have been found affecting body composition and meat quality. These include the Halothane (*HAL*) or stress gene (Christian 1972; Fujii et al. 1991), the Rendement Napole (*RN*) or acid meat gene (Monin and Sellier 1985; LeRoy et al. 1990; Milan et al. 2000; Ciobanu et al. 2001), the Heart-fatty acid binding protein (*H-FABP)* gene, and the Fatty acid binding protein (*A-FABP)* gene (Gerbens et al. 1998).

Malek et al. (2001 a, b) detected several QTL for meat quality traits (24-hr loin pH, 24-hr loin Minolta, 48-hr loin Minolta) and body composition traits (average backfat, lumbar, last rib backfat) on chromosome 5 in the pig. These results make this chromosome attractive for a positional candidate gene approach to eventually find the causative gene(s) associated with these QTL. Estimated position of most QTL was at the distal marker on this chromosome (SW378). Therefore it was decided that, along with a positional candidate gene analysis, adding more markers at the end of chromosome, to extend the map coverage for this chromosome, will offer a better picture of the location of QTL.

The main objective of this study was to perform a positional candidate gene analysis in a region on porcine chromosome 5 (SSC5) by investigating the following two genes that were selected based on human comparative mapping and their functions. These genes were the ATPase, Ca^{2+} transporting, plasma membrane 1 gene (***ATP2B1***) and the dual specificity phosphatase 6 gene (***DUSP6***). The mapping of these genes in the pig genome will also increase or confirm regions of synteny between pigs and humans.

Human *ATP2B1* is expressed in neuronal tissue and skeletal muscle and plays an important role in the fine-tuning of the intracellular concentration of Ca^{2+} (Gromadzinska et al. 2001). This enzyme exhibits a high degree of tissue specificity and is regulated by several mechanisms. Protein phosphatases decrease both the basal activity of Ca^{2+}-ATPase and its affinity for calmodulin. *ATP2B1* isoforms are encoded for by at least four separate genes and the diversity of these enzymes is further increased by alternative splicing of transcripts (Olson 1991).

The protein encoded by *DUSP6* is a member of the dual specificity protein phosphatase subfamily. These phosphatases inactivate their target kinases by dephosphorylating both the phosphoserine/threonine and phosphotyrosine residues (Smith et al. 1997). They negatively regulate members of the mitogen-activated protein (MAP) kinase superfamily, which are associated with cellular proliferation and differentiation. Different members of the family of dual specificity phosphatases show distinct substrate specificities for various MAP kinases, different tissue distributions and subcellular localization, and different modes of how and when they are expressed by extracellular stimuli (Toyota et al. 2000).

Materials and Methods

Animals and phenotypic data

Two groups of animals were used in this study. The first was a three generation resource family of a cross between animals from the Berkshire and Yorkshire (BxY) breeds (Malek et al. 2001a). The F_2 generation of this family was used here for further QTL linkage analysis and for an association study. In this cross, the Berkshire breed was chosen as it is regarded as having very good meat quality. We also used data from five different pig commercial lines (PIC) for independent association studies. These lines are described as: line "A" Landrace, line "B" Large White, line "C" Duroc x Large White, line "D" a synthetic line created from several different synthetic populations, and line "E" Berkshire.

Phenotypic measures for growth, body composition and meat quality traits from the BxY family were collected as described by Malek et al. (2001 a, b). A total of 29 traits were measured in the commercial populations for growth, fat content, meat quality (color

score, marbling, firmness, Minolta and Hunter light reflectance, ham and loin muscle pH), glycogen content, body composition at a commercial packing plant and individual meat color (loin and ham reflectance) and individual loin and ham pH 24-hrs after harvest were obtained. Backfat depth and muscle depth were measured by ultrasound prior to slaughter. The Hennessy grading probe was used to measure fat thickness and muscle depth on the carcass (Henessey probe backfat, Henessey probe rib thickness, and Henessey probe loin depth).

Tissue Samples and DNA Isolation.

Blood, tissue samples and phenotypes were collected and recorded on the BxY family as described in Malek et al. (2001a). Blood samples from five closed commercial lines of pigs were obtained using approved animal care methods. Genomic DNA was isolated from whole blood by standard salting out procedures according to manufacturer instructions (GIBCO/BRL, Rockville, MD).

PCR and Polymorphism Discovery.

Primer set "A", as reported by Farber et al. (2001), was used to amplify a fragment of the *DUSP6* gene, including part of exon 3 based on the human gene organization (Table 1). Primer set "D" was designed based on human sequences (GenBank accession no: L14561) to amplify *ATP2B1* and is presented in Table 1.

The PCR reactions were performed using 12.5 ng of porcine genomic DNA, an optimum concentration of $MgCl_2$ (Table1), 0.2 m*M* dNTP, 0.25 μ*M* of each primer, 0.35 U *Taq* DNA polymerase (Promega, Madison, WI) and PCR buffer (10 m*M* Tris-HCl, 50 m*M*

KCl, and 0.1% Triton®X-100) in a 10µl final volume. The cycling conditions included initial denaturation at 94° C for 3 min, followed by 30 cycles at 93° C for 45 s, optimum annealing temperature for 45 s (Table1), 72° C for 1 min and final extension at 72° C for 10 min.

The PCR products from several individuals from each of the commercial breeds of pigs were directly sequenced using dye terminators and an ABI 377 sequencer (Perkin Elmer, Foster City, CA). We used Sequencher software (Gene Codes Corporation, version 4.0.5, Ann Arbor, MI) to assemble the sequences and to identify polymorphisms. Sequences were compared with human sequence for similarity.

Polymorphisms and Restriction Fragment Length Polymorphism (RFLP) tests

Single nucleotide polymorphisms (SNPs) were discovered within *ATP2B1* and *DUSP6* that affect the recognition sequence for the *Afl*III and *Pst*I restriction enzymes, respectively. Primer set "E" was used to amplify a fragment of the *ATP2B1* gene that contains the *Afl*III polymorphism. A PCR product that includes the *DUSP6-Pst*I marker was generated using the "B" primer set. After amplification, both PCR products were subjected to overnight digestion, at 37°C. The digested fragments were separated by electrophoresis using 2.5% NuSieve agarose (FMC, Rockland, ME). Ethidium bromide staining was used for DNA visualization.

Genetic linkage and QTL mapping.

The *DUSP6* and *ATP2B1* genes were mapped in the BxY family linkage map using the CRI-MAP (version 2.4) mapping program (Green et al. 1990). Besides the markers listed in

Malek et al. (2001a), two additional genetic markers (*IGF1* and SW1954) were included in the analysis. Two-point linkage analysis was performed in order to place genes into linkage groups and the best order was determined with multipoint linkage analysis (Green et al. 1990). The interval mapping method (Haley et al. 1994) was then used as described in Malek et al. (2001a,b) to confirm QTL for meat quality on SSC 5. The QTL effects were estimated and represent the average Berkshire allele effect compared to the average Yorkshire allele effect. The F-statistic thresholds for the 5% chromosome-wise and the 5 and 1% genome-wise levels were determined by permutation test.

Association studies

Berkshire x Yorkshire F_2 population analysis.

An analysis of variance procedure was used to test associations of *ATP2B1-Afl*III and *DUSP6-Pst*I genotypes with meat quality and body composition traits using the SAS general linear model computer program (SAS/STAT, 1990). Besides *ATP2B1-Afl*III or *DUSP6-Pst*Igenotype, which were initially analyzed separately, the model accounted for dam, slaughter date, and sex. Sire was not used as it was confounded with slaughter date. Live weight at slaughter was added in the model as a covariable for average backfat, tenth rib backfat, lumbar backfat, last rib backfat, loin eye area, carcass weight, carcass length, total lipid, marbling, and cholesterol. The analysis was also run with both candidate genes included in the model and with and without inclusion of an interaction term. Least squares (LS) means were estimated for all three genotypic classes for both genes.

Commercial lines analyses.

Associations of the *ATP2B1-Afl*III and *DUSP6-Pst*I genotypes with body composition and meat quality traits were tested separately for each line using a mixed model procedure (SAS/STAT, 1990) based on a model that included sire as a random effect and slaughter date and genotype(s) as fixed effects. Since the *ATP2B1* gene was informative only in the C line and the *DUSP6* gene only in lines C and D, analyses could be conducted only for those lines. For the *DUSP6* gene, the data were also used for an across line analysis, where line was included as fixed effect in the model. A model including both genes as fixed effects with and without an interaction term was also run for line C. LS means were estimated for all three genotypic classes within the commercial lines for each of the polymorphisms analyzed individually.

The combined effects of the *ATP2B1-Afl*III and *DUSP6-Pst*I polymorphisms were also estimated as an haplotype substitution effect. Contrasts between haplotypes were estimated from a model that included sire as random effect and slaughter day and one variable per haplotype, with values –1, 0 and 1 corresponding to the number of copies (0, 1 and 2) of the haplotype. The four possible haplotypes were denoted as follows: hap1 = *DUSP6* allele1_ *ATP2B1* allele1, hap2 = *DUSP6* allele1_ *ATP2B1* allele2, hap3 = *DUSP6* allele2_ *ATP2B1* allele1, hap4 = *DUSP6* allele2_ *ATP2B1* allele2. Only haplotypes 2, 3, and 4 were observed in the C line.

Results

Identification of polymorphisms and linkage mapping

The PCR product for *ATP2B1* and *DUSP6* was sequenced and the sequence showed 94% and 84% similarity to human *ATP2B1* and *DUSP6* respectively.

An SNP was discovered in intron 17 of the *ATP2B1* gene. The SNP was confirmed by enzyme digestion using PCR-RFLP. This polymorphism is a synonymous G to A substitution and determines a restriction site for the *Afl*III enzyme. The fragments sizes generated by the digestion of the 730 bp fragment were a 330bp fragment (allele 1), and 164bp and 166bp fragments (allele 2). There was also a 400bp monomorphic fragment for all products.

An SNP for *DUSP6* was confirmed by enzyme digestion by PCR-RFLP. This polymorphism (a C to G substitution) determines a restriction site for the enzyme *Pst*I. The fragments sizes generated by the digestion of the 990bp fragment were a 820bp fragment (allele 1), and 723bp and 97bp fragments (allele 2). There was a 170bp monomorphic fragment for all products.

In the BxY family, segregation of the *Afl*III and *Pst*I polymorphisms followed Mendelian autosomal inheritance patterns. The *ATP2B1* gene mapped to SSC5, with significant pair-wise linkage to six markers (Figure 1). The gene order was (recombination frequency, LOD score): *DUSP6* (0.12, 27.45), *IGF1* (0.15, 35.86), SW1954 (0.04, 7.18), SW378 (0.01, 96.25), SW995 (0.12, 47.40) and SW904 (0.26, 14.95).

Porcine *DUSP6* mapped to SSC5, with significant pair-wise linkage to six markers: *ATP2B1* (0.12, 27.45), *IGF1* (0.21, 32.12), SW1954 (0.11, 62.37), SW378 (0.11, 40.85), SW995 (0.18, 31.33) and SW904 (0.33, 7.96). These results and multipoint linkage

analysis show that the *ATP2B1* and *DUSP6* genes are located at the end of the linkage map of SSC5 (Figure 1). After adding *ATP2B1, DUSP6* and the other two markers (*IGF1* and *SW1954*), order of the other markers was the same as in Malek et al. (2001a).

QTL mapping

Previous QTL analyses by Malek et al. (2001a,b) detected several significant QTL in the distal region of SSC5 (Figures 1 of Malek et al. 2001a,b). These included QTL for meat quality traits (24-hr loin pH, 24-hr loin Minolta, 48-hr loin pH, 48-hr loin Minolta, 48-hr loin hunter) and body composition (average backfat, last rib backfat, lumbar backfat). Re-analysis of these QTL with the inclusion of the two candidate genes and the additional two markers (Figure 2,3) caused some changes in the F values of the peaks on SSC5 compared to Malek et al. (2001a,b). In particular, F-values increased from 7.35 to 11.63 for average backfat, from 9.51 to 11.24 for last rib backfat, and from 7.25 to 11.87 for lumbar backfat. Three more QTL (F value, position), for tenth rib backfat (5.55, 125), average drip loss (5.32, 35), and water holding capacity (6.28, 122), were detected in this study, which were not reported by Malek et al. (2001a,b). Compared to findings in Malek et al. (2001a, b), the most likely position of QTL detected on distal SSC5 were shifted toward the interval between SW1954 and *DUSP6*.

Berkshire x Yorkshire F_2 association study

Frequencies of allele 1 in the Berkshire and Yorkshire grandparents of the BxY family were 0.25 and 0.67 for *DUSP6* and 0.0 and 0.22 for *ATP2B1*. The frequency of allele 1 in the total F_2 BxY population was 0.52 for *DUSP6* and 0.19 for *ATP2B1*.

Significant results for the association analysis in the BxY family are shown in Table 3. There were significant effects of the *ATP2B1-Afl*III genotypes on loin eye area, carcass length, marbling, average daily gain on test at the $p<0.01$ level and on tenth rib backfat, average glycogen content, average glycolytic potential, and 24-hr ham and loin pH at the $p<0.1$ level. The single gene association analyses with *DUSP6-Pst*I genotypes showed significant effects on body composition (lumbar backfat and average back fat) and growth traits (16-days weight, average daily gain to weaning, and average daily gain on test).

A joint analysis including both genes as fixed effects but without interaction revealed significant effects for *ATP2B1-Afl*III genotypes on loin eye area, marbling, average daily gain on test, average glycogen, average glycolytic potential, and 24 –hr ham pH (Table 4). In the case of *DUSP6-Pst*I genotypes, there were some moderate to strong effects on average lactate, lumbar backfat, 16-day weight, average daily gain to weaning, average daily gain on test, and 24-hr loin pH. (Table 4). These results were similar to those obtained from the single gene model. However, when both genes were fitted simultaneously, 24-hr loin pH had a weak association with *DUSP6* instead of with *ATP2B1* in the single gene analyses. The inconsistency of these results might suggest that the QTL found for 24-hr loin pH on SSC5 is not caused by the specific polymorphisms in the *DUSP6* or *ATP2B1* genes, but rather by another mutation or a closely linked gene.

The results of analysis (Table 5) of the interaction between the two genes revealed significant interactions ($P<0.06$) on last rib backfat, average backfat, and average daily gain to weaning. For these a very interesting interaction was noted. When the *ATP2B1* genotype was 11 or 12, the greater fatness were associated with the *DUSP6* 11 genotype. However, this was reversed when *ATP2B1* was 22, for which the fattest pigs were the *DUSP* 22

genotype. This suggests that these special combinations defined an underlying mutation in a third gene.

Commercial line association study

The *ATP2B1-Afl*III and *DUSP6-Pst*I polymorphisms were tested in 100-200 randomly selected individuals from each of five commercial lines. Lines C and D were informative and used for further analysis of *DUSP6*. The frequency of allele 1 for the *DUSP6* gene was 0.34 in line C and 0.48 in line D. Only line C was informative for the *ATP2B1* marker and the frequency of allele 1 was 0.30 for this line.

The *ATP2B1-Afl*III genotypes were not significantly associated with any of the body composition traits in line C. However, the *ATP2B1-Afl*III genotypes had a significant association with 24-hr ham Minolta ($P < 0.10$) (Table 6). There were significant differences between the LS means for the 12 and 22 genotypes at $p<0.05$, with the 22 genotype having the better color reflectance.

The *DUSP6-Pst*I genotypes were associated with light reflectance (loin Minolta), and body composition (Henessey probe backfat thickness) in two commercial lines (C with $p<0.10$ and D with $p<0.01$) (Table 7). Significant effects for *DUSP6* were also revealed for 24-hr loin pH ($p<0.1$) and Henessy probe loin depth ($p<0.05$) in line D but not in line C (Table 7). There were significant differences between the LS means of the *DUSP6-PstI* genotypes, with the 11 genotype being the fattest. Except for Loin Minolta in line C, differences were significant between all *DUSP6* genotypes for all traits.

The results of joint analysis of lines C and D revealed significant differences ($p<0.05$) between *DUSP6-Pst*I genotypes only for Henessey probe backfat thickness (Table 7). This confirms that the *DUSP6-Pst*I polymorphism is associated with backfat.

For the haplotype analysis in line C, three haplotypes were present. Effects of the haplotypes were estimated as deviations from haplotype 4, which was set to zero. Haplotype 2 was the most frequent in this line. Analysis revealed that, compared to haplotype 4, haplotype 3 was associated with significantly higher reflectance ($p<0.01$), higher carcass weight ($p<0.1$), and less back fat when measured on the carcass ($p<0.01$), but with more fat ($p<0.1$) when measured by ultrasound on the live animal (Table 8). Clearly with both the interaction analysis and the haplotype analysis some traits are significant.

Discussion

Linkage analysis was used to localize the *ATP2B1* and *DUSP6* genes to SSC5. Human *ATP2B1* and *DUSP6* were previously physically mapped to human chromosome 12q21-q23 (Olson 1991) and 12q22-q23 (Smith et al. 1997), respectively. The mapping results of this study are in accordance with the results obtained by chromosomal painting between human and pig (Goureau et al. 1996). These results also confirm our earlier finding regarding the break point of human chromosome 12 (Chapter 4).

Malek et al. (2001a,b) reported several QTL for body composition and meat quality on the distal arm of SSC5 in the BxY population that was used in this study. By extending the marker map, this study identified several additional QTL (for tenth rib backfat, average drip loss, and water holding capacity) which had not been reported in the previous study.

Markers were added distal to SW378, which helped to extend the map coverage for this chromosome and allowed a better picture of the location of QTL in the distal region of SSC5.

ATP2B1 gene

The analysis of the BxY F_2 population showed evidence of associations between *ATP2B1-AflIII* genotypes and 24 hr loin and ham pH traits (Table 3). QTL analysis showed QTL for loin pH but not for ham pH (Table 2). The ability to detect QTL using the breed-cross linkage analysis depends on differences in QTL allele frequencies between the parental breeds. Gene frequencies for the *ATP2B1-AflIII* polymorphism were not very different between the Berkshire and Yorkshire grand parents (0.0 and 0.22). Therefore, if *ATP2B1-AflIII* is in tight linkage disequilibrium with the causative mutation, QTL analysis could have limited power to detect QTL for ham pH.

The BxY F_2 resource family is a result of a cross between two divergent breeds and as a result large linkage disequilibrium is expected. Therefore, it was necessary to investigate and confirm the effects of the *ATP2B1-AflIII* polymorphism in commercial lines. The results of the only line that was polymorphic (line C) showed evidence of an association between the *ATP2B1* genotypes and light reflectance (24-hr ham Minolta, $P<0.01$), with the 22 genotype having lower light reflectance, which results from darker meat (Table 6). The QTL analysis showed a QTL for this trait near this gene.

The ham and loin pH are correlated with light reflectance such as Minolta (Cameron, 1990; Huff-Lonergan et al. 2001). Therefore finding an association in line C

with lower 24-hr ham Minolta for 22 genotype could be related to the results from the BxY population, which showed that the 22 genotype had higher pH.

The *ATP2B1-Afl*III polymorphism was informative in only one commercial line. More informative polymorphisms in the *ATP2B1* gene must be found to test associations in other commercial lines.

DUSP6 gene

The results of BxY F_2 population (Table 3) and lines C and D (Table 7) confirmed there is an association between the *DUSP6-Pst*I polymorphism and body composition traits. These results also confirmed QTL found for body composition at the end of the SSC5 q-arm (Table 2). The *DUSP6* gene is associated with cellular proliferation and differentiation (Toyota et al. 2000). This biological effect of *DUSP6* gene is in line with the findings from this study.

Although some growth related traits are shown to be associated *DUSP6-Pst*I genotypes with the BxY F_2 population, we were not able to confirm these associations in the commercial lines. There was also no QTL reported for growth related traits in this region, thus the association of *DUSP6-Pst*I with growth in the BxY population may be a false positive result.

Associations observed in the commercial lines shows that *DUSP6-Pst*I has associations with some meat quality related traits (light reflectance and pH related traits, Table 7), but this was not confirmed by the association analysis in the BxY F_2 population (Table 6). Although QTL reported for meat quality (light reflectance and pH related traits)

are 10 cM from *DUSP6,* there is not enough evidence to verify if these traits are associated with the *DUSP6* or *ATP2B1* genes.

Haplotype associations

Haplotypes 3 and 4 have a common *DUSP6* allele and different in *ATP2B1* allele. We also found that these results show that the *ATP2B1* gene is associated with ham Minolta (a and b) trait. We also know that haplotypes 2 and 4 are the different for the *DUSP6* allele and the same for the *ATP2B1* allele. The haplotype analysis considering these two haplotypes revealed some effects for fatness traits. The results for backfat thickness (lower backfat) were in line with results of haplotype with the lean meat percentage of the carcass (more body mass) and backfat depth (smaller fat depth). Therefore, these results show that the *DUSP6* gene is associated with body composition trait. There were also some inconsistent results ($p<0.1$) for *DUSP6* (lab Minolta) and *ATP2B1* (probe rib thickness and backfat depth), which can not be explained by haplotype analysis.

Summary

The detected QTL for body composition and meat quality traits explained 2.3% to 5.3% of the phenotypic variance for the individual traits in the F_2 population, respectively (Table 2). The Berkshire breed is generally regarded as having very good meat quality, but our results indicated that Yorkshires alleles had lower backfat, drip loss, water holding capacity, light reflectance, and higher pH and were superior for body composition and meat quality for chromosome 5 (Table 2). Therefore, use of QTL in marker assisted selection could result in substantial improvements.

Considering the several significant association results observed here for meat quality and body composition, the identified *ATP2B1* and *DUSP6* polymorphisms could potentially be used as a markers in order to track these QTL and to discover the differences in DNA sequences responsible for the phenotypic variation.

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Table 1. The primer sequences and PCR conditions for *DUSP6* and *ATP2B1*.

Gene	Primer Set	Primers (5'- - 3')[a]	PCR conditions[b]		
			bp	T	{$MgCl_2$}
DUSP6	A	F TGC AGG TCT CTA CGT TCC AA R GCC AAG CAA TGC ACC AAG	990	55° C	3.75
DUSP6	B	F AGA CCC TTG CAG TCT GCT TG R GCC AAG CAA TGC ACC AAG	389	55° C	3.75
DUSP6	C	F TGC AGG TCT CTA CGT TCC AA R TACCCTCGCCGTCTCACTGCTA	373	55° C	3.75
ATP2B1	D	F AGA GGG CTG GAA TTA CTG TGC R CCA GTG TAT GCT TAT CAG TAG G	940	61° C	1.5
ATP2B1	E	F AAT AAG CCT CTC ATC TCA CGC A R AAA GTG CCT AAA ACA ATT GTG C	730	61° C	1.5
ATP2B1	F	F ACG CAA TAA AGA AAT GTC AGA G R ACC AGT GTA TGT TTG TCA GTA G	940	61° C	1.5
ATP2B1	J	F CAG ATG ACA ACT TTA CGA GCA R GCG TGA GAT GAG AGG CTT ATT	750	61° C	1.5
ATP2B1	K	F AAT AAG CCT CTC ATC TCA CGC A R AAA GTG CCT AAA ACA ATT GTG C	516	61° C	1.5

[a] F: Forward, R: reverse

[b] bp: base pairs; T: optimal annealing temperature.

Table 2. Evidence for significant QTL at the 5% chromosome-wise level for various meat quality traits for pig chromosome 5.

Trait	F-Value[a]	Location (cM)	Additive Effect[b]	Additive S. E.	Dominance Effect	Dominance S. E.	Variance[c] (%)
Average backfat (cm)	11.63	122	0.166	0.035	0.046	0.050	5.02
Last rib backfat (cm)	11.24	120	0.166	0.036	0.058	0.052	5.02
Lumbar backfat (cm)	11.87	120	0.196	0.043	0.117	0.063	5.37
Tenth rib backfat (cm)	5.55	125	0.133	0.041	-0.057	0.061	2.38
24-hr Loin Minolta	6.90	130	0.619	0.283	-1.293	0.437	3.65
24-hr Loin pH	6.00	126	-0.024	0.011	0.039	0.015	2.68
48 hr Loin pH	6.55	85	-0.042	0.012	0.002	0.019	3.52
48-hr Loin Minolta	6.91	125	0.496	0.198	-0.778	0.288	3.07
48-hr Loin Hunter	6.38	125	0.476	0.207	-0.809	0.301	2.85
Average drip loss (%)	5.32	35	0.422	0.130	0.028	0.211	2.90
Water holding capacity (g)	6.28	122	0.020	0.008	-0.026	0.011	2.78

[a] The chromosome-wise F-statistic threshold at the 5% level, as determined by permutation test, was 5.00.

[b] Additive (a) and dominance (d) QTL effects correspond to genotype values of +a, d, and –a , respectively, for individuals having inherited two Berkshire alleles, heterozygotes, and individuals with two Yorkshire alleles. Positive additive effects indicate that Berkshire alleles increased the trait, negative that the Berkshire alleles decreased it. Dominance effects are relative to the mean of the two homozygotes.

[c] % Variance = genetic variance at the QTL based on estimated additive and dominance effects and allele frequencies of ½, as a percent of the residual variance in the F_2.

[d] units of measure - μmol/g

* Significant at the 5% genome-wise level (F>7.05)

** Significant at the 1% genome-wise level (F> 8.88)

Table 3. Summary of association analysis results for the *ATP2B1-AflIII* and *DUSP6-PstI* genotypes in the Berkshire x Yorkshire 3-generation family.

Gene	Trait	p value	LS means (s.e.)[a]		
			11	12	22
	Length (cm)	0.01	84.75 (0.57)a	84.53 (0.19)e	83.94 (0.14)bf
	Loin eye area (cm^2)	0.03	38.99 (1.22)c	36.00 (0.41)da	35.67 (0.30)db
	Marbling (1-5)	0.01	3.59 (0.18)a	3.56 (0.06)e	3.75 (0.04)bf
ATP2B1	Tenth rib backfat (cm)	0.16	3.12 (0.14)a	3.25 (0.05)ba	3.31 (0.04)b
	Average daily gain on test (kg/day)	0.05	0.68 (0.01)a	0.68 (0.004)c	0.69 (0.003)db
	Average glycogen (μmol/g)	0.08	112.95 (4.57)a	106.57 (1.55)b	104.03 (1.13)b
	Average glycolytic potential (μmol/g)	0.07	10.18 (0.93)a	9.58 (0.32)e	8.56 (0.23)bf
	24-hr ham pH	0.09	5.77 (0.06)a	5.86 (0.02)bc	5.89 (0.02)db
	24-hr loin pH	0.19	5.67 (0.05)a	5.76 (0.02)b	5.75 (0.01)b
	Carcass weight (kg)	0.07	86.81 (0.21)ca	87.37 (0.16)d	87.19 (0.25)b
DUSP6	Lumbar backfat (cm)	0.02	3.43 (0.06)c	3.58 (0.05)da	3.66 (0.07)db
	Average backfat (cm)	0.17	3.20 (0.05)c	3.29 (0.40)d	3.34 (0.06)d
	16-day weight (kg)	0.01	5.17 (0.11)	4.92 (0.08)a	4.67 (0.17)b
	Average daily gain to weaning(kg/day)	0.004	0.25 (0.006)	0.23 (0.005)a	0.22 (0.01)b
	Average daily gain on test (kg/day)	0.09	0.69 (0.05)a	0.69 (0.003)e	0.67 (0.01)bf

[a] Least squares means for three genotype classes were estimated for each trait and are presented with standard errors of the estimates in parenthesis. Significant differences between least squares estimates are indicated by 2-letter combinations : a-b $p<.5$, c-d $p<.05$, e-f $p<.005$. An estimate with letter "a" is significantly different at $p<.05$ from estimate (s) with letter "b", same for c-d and e-f at their respective significance levels.

Table 4. Significant effects of the *ATP2B1* and *DUSP6* genotypes in the Berkshire x Yorkshire population when both are fitted in the model with no interaction.

Gene	Trait	p value	LS means (s.e.) [a]		
			11 [b]	12	22
ATP2B1	Loin eye area (cm^2)	0.07	38.68 (1.28)c	35.91 (0.43)d	35.70 (0.32)d
	Marbling (1-5)	0.01	3.58 (0.19)	3.53 (0.06)e	3.73 (0.04)f
	Average daily gain on test (kg/day)	0.01	0.67 (0.01)	0.67 (0.01)e	0.69 (0.003)f
	Average glycolytic potential (μmol/g)	0.05	113.59 (4.80)ac	107.76(1.6)bc	104.36 (1.2)d
	Average glycogen (μmol/g)	0.03	10.24(0.99)ac	9.65 (0.34)a	8.74 (0.25)d
	24-hr ham pH	0.09	5.77 (0.06)ac	5.86 (0.02)ba	5.89 (0.02)db
	Average lactate (μmol/g)	0.01	86.36 (1.71)ce	90.15 (1.5)da	91.87 (1.9)fb
DUSP6	Lumbar backfat (cm)	0.02	3.43 (0.08)c	3.58 (0.07)d	3.63 (0.09)d
	16-day weight (kg)	0.02	5.17 (0.11)ae	4.92 (0.08)ba	4.67 (0.17)fb
	Average daily gain to weaning (kg/day)	0.001	0.25 (0.009)ae	0.23 (0.01)ba	0.22 (0.01)fb
	Average daily gain on test (kg/day)	0.03	0.69 (007)a	0.69 (0.006)a	0.67 (0.007)b
	24-hr loin pH	0.19	5.86 (0.05)a	5.84 (0.03)a	5.81 (0.03)b

[a] Least squares means for three genotype classes were estimated for each trait and are presented with standard errors of the estimates in parenthesis. Significant differences between least squares estimates are indicated by 2-letter combinations : a-b $p<.5$, c-d $p<.05$, e-f $p<.005$. An estimate with letter "a" is significantly different at $p<.05$ from estimate (s) with letter "b", same for c-d and e-f at their respective significance levels.
[b] Three genotype classes.

Table 5. Significant effects of *ATP2B1* and *DUSP6* genotypes in the Berkshire x Yorkshire population when both are fitted in the model with interaction.

	p value			LS means[a]								
				11[b]			12			22		
	ATP2B1	*DUSP6*	Interaction	11[c]	12	22	11	12	22	11	12	22
Last rib backfat (cm)	0.81	0.35	0.03	3.99 (0.39)c	2.99 (0.18)d	3.00 (0.54)	3.18 (0.08)	3.19 (0.08)	3.15 (0.11)	2.98 (0.08)ec	3.23 (0.05)f	3.24 (0.07)d
Average backfat (cm)	0.95	0.80	0.06	3.62 (0.37)a	3.12 (0.17)b	2.99 (0.51)b	3.29 (0.07)	3.25 (0.07)	3.29 (0.10)	3.10 (0.07)e	3.36 (0.04)f	3.40 (0.70)f
Average daily gain to weaning (kg/day)	0.21	0.01	0.06	0.36 (0.05)c	0.22 (0.02)d	0.26 (0.07)	0.25 (0.01)c	0.23 (0.01)d	0.23 (0.01)d	0.25 (0.01)c	0.24 (0.01)c	0.21 (0.01)d

[a] Least squares means were estimated for each trait and are presented with standard errors of the estimates in parenthesis.
Significant differences between least squares estimates are indicated by 2-letter combinations : a-b $p<.5$, c-d $p<.05$, e-f $p<.005$. An estimate with letter "a" is significantly different at $p<.05$ from estimate (s) with letter "b", same for c-d and e-f at their respective significance levels.
[b] Three genotype classes for *ATP2B1* [c] Three genotype classes for *DUSP6*

Table 6. Significant association results for the *ATP2B1 Aff*III genotypes in the C commercial pig line.

Trait	Mean (s.e.)	p value	LS means (s.e.)[a]		
			11	12	22
24-hr ham Minolta	48.49 (0.24)	0.09	48.40 (1.03)	48.80 (0.42)c	47.67 (0.37)d
			15[b]	100	152
Dirty weight of the carcass[c] (kg)	111.59 (0.52)	0.09	111.27 (1.82)	109.5 (1)c	111.9 (0.95)d
			33	139	195

[a] Least squares means for three genotype classes were estimated for each trait and are presented with standard errors of the estimates in parenthesis. Significant differences between least squares estimates are indicated by 2-letter combinations : a-b $p<.5$, c-d $p<.05$, e-f $p<.005$. An estimate with letter "a" is significantly different at $p<.05$ from estimate (s) with letter "b", same for c-d and e-f at their respective significance levels.

[b] Number of individuals for this genotype.

[c] Carcass weight right after slaughter (without head and blood)

Table 7. Significant association results of *DUSP6 PstI* genotypes in two commercial pig lines.

Line	Trait	Mean (s.e.)	p value	LS means (s.e.)[a]		
				11	12	22
D	24-hr loin Minolta	45.34 (0.20)	0.09	44.57 (0.47)[c]	45.64 (0.39)da	45.12 (0.46)b
				52[b]	96	66
D	24-hr loin pH	5.74 (0.01)	0.07	5.76 (0.02)ac	5.72 (0.02)b	5.69 (0.02)ad
				52	97	66
D	Henessey probe backfat thickness (mm)	13.68 (0.20)	0.36	13.84 (0.49)a	14.41 (0.39)b	13.79 (0.47)a
				46	84	60
D	Henessy probe loin depth (mm)	63.40(0.60)	0.05	63.96 (1.44)a	62.16 (1.13)bc	65.85(1.36)bd
				46	84	60
C	24-hr loin Minolta	46.51 (0.16)	0.04	46.14 (0.50)	45.70 (0.29)c	46.55 (0.31)d
				52	224	208
C	Henessey probe backfat thickness (mm)	15.70 (0.16)	0.01	16.78 (0.49)e	15.34 (0.29)f	15.18 (0.31)f
				52	224	210
C+D	Henessey probe backfat thickness (mm)	15.13 (0.13)	0.05	15.46 (0.35)ce	14.80 (0.24)d	14.54 (0.27)f
				98	308	270

[a] Least squares means for three genotype classes were estimated for each trait and are presented with standard errors of the estimates in parenthesis. Significant differences between least squares estimates are indicated by 2-letter combinations : a-b $p<.5$, c-d $p<.05$, e-f $p<.005$. An estimate with letter "a" is significantly different at $p<.05$ from estimate (s) with letter "b", same for c-d and e-f at their respective significance levels.
[b] Number of individuals for this genotype.

Table 8. Haplotype [a]frequencies, estimates and contrast p values for the *Pst*I and *Afl*III genotypes in the *ATP2B1* and *DUSP6* genes in the C commercial pig line.

Trait	Mean (s.e.)	Haplotype Frequency			Haplotype Estimate			Contrast P Value		
		hap 2	hap 3	hap4	hap 2	hap 3	hap4	2 vs 3	2 vs 4	3 vs 4
24-hr loin minolta (l)	46.66 (0.19)	0.32	0.30	0.39	-0.54	0.34	0	0.01	0.10	0.30
24-hr loin minolta (a)	6.30 (0.08)	0.32	0.30	0.39	0.26	0.14	0	0.41	0.06	0.32
24-hr ham minolta (l)	48.63 (0.25)	0.35	0.24	0.41	0.11	1.31	0	0.02	0.81	0.01
24-hr ham minolta (b)	8.08 (0.11)	0.35	0.24	0.41	0.15	0.41	0	0.22	0.40	0.03
Henessey probe backfat thickness (mm)	15.77 (0.19)	0.32	0.30	0.38	0.85	-0.01	0	0.01	0.01	0.98
Henessey probe rib thickness (mm)	15.3 (0.30)	0.37	0.24	0.39	-0.85	-1.27	0	0.52	0.10	0.03
Lean meat percentage of the carcass	45.45 (0.11)	0.37	0.24	0.39	-0.37	-0.04	0	0.13	0.03	0.83
Backfat depth (mm)	13.49 (0.19)	0.32	0.30	0.38	0.70	0.5	0	0.54	0.02	0.10
Carcass weight at the end of test (kg)	110.7 (0.46)	0.28	0.32	0.39	1.25	-1.12	0	0.94	0.08	0.09

[a] hap1 = *DUSP6* allele1_ *ATP2B1* allele1, hap2 = *DUSP6* allele1_ *ATP2B1* allele2 , hap3 = *DUSP6* allele2_ *ATP2B1* allele1, hap4 = *DUSP6* allele2_ *ATP2B1* allele2

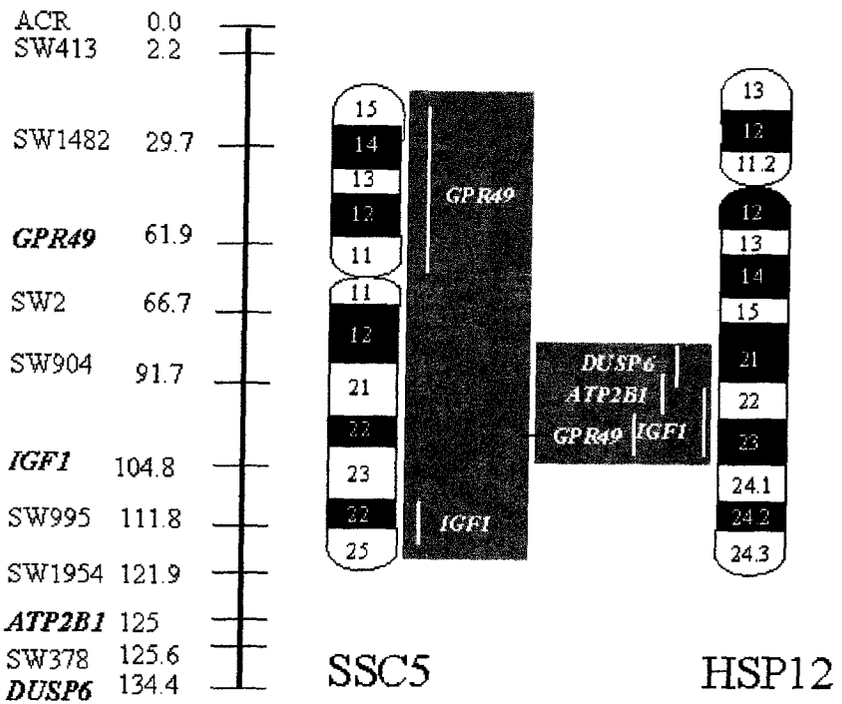

Figure 1. Comparative physical and linkage map, of pig chromosomes 5 with human chromosome 12. The shaded regions summarize results of bi-directional painting (Goureau et al. 1996).

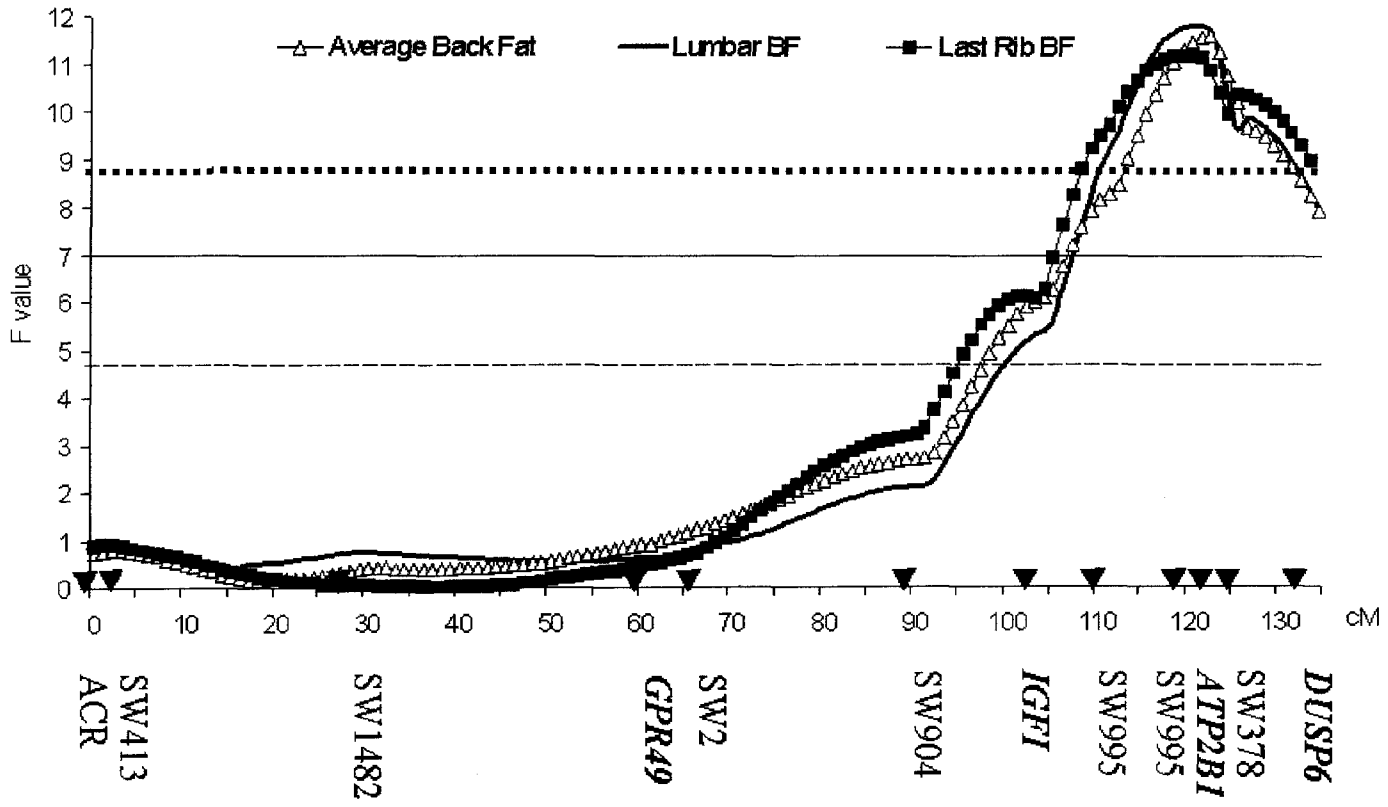

Figure 2. F-ratio curves for evidence of QTL associated with meat quality for SSC 5. The x-axis indicates the relative position on the linkage map. The y-axis represents the F-ratio. Arrows on the x-axis indicate the position where a marker was present. Three lines are provided for 5% chromosome-wise (------), 5% genome-wise (——) and the 1% genome-wise (.....) significance.

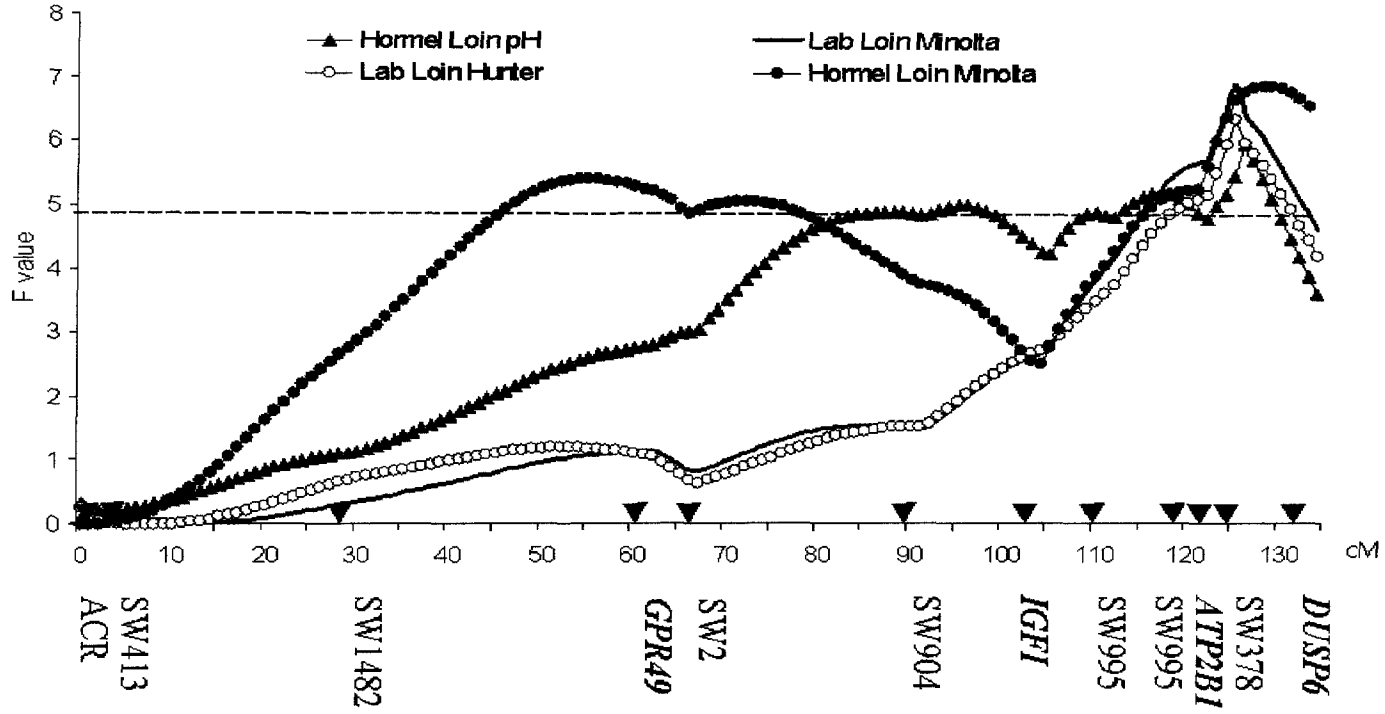

Figure 3. F-ratio curves for evidence of QTL associated with meat quality for SSC 5. The x-axis indicates the relative position on the linkage map. The y-axis represents the F-ratio. Arrows on the x-axis indicate the position where a marker was present. A line is provided for 5% chromosome-wise (------).

CHAPTER 6. LINKAGE AND PHYSICAL MAPPING OF THE PORCINE PREPRO-OREXIN GENE.

A paper published in Mammalian Genome[1]

Massoud Malek[2], Stefan Marklund[2], Cheryl Dyer[3], Robert Matteri[3], and Max Rothschild[2]

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number AF169352.

It is well established that the brain, and specifically the hypothalamus, is a major site where various central nervous system signals are integrated to affect the expression of complex hormonal and neuroendocrine functions, such as food intake and energy homeostasis. Orexin-A and B (also called prepro-orexin) are hypothalamic peptides, encoded by a single mRNA transcript, which are derived from the same precursor. These peptides bind and activate two closely related orphan G protein-coupled receptors (Sakurai et al. 1998). Prepro-orexin has been proposed to have a physiological role in the regulation of food intake in the mouse, rat, pig, and human (Mondal et al. 1999). Edwards et al. (1999) studied the effect of orexin-A and B on feed intake in the rat. Their data indicated that orexin-A consistently stimulated food intake, but orexin-B only occasionally stimulated food intake in the rat. Dyer et al. (1999) showed that cumulative feed intake increased by administration of orexin-B and total feed intake at 24 h was increased by 18% in orexin-treated pigs. Given the role of

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prepro-orexin, the present study was designed to characterize gene structure and chromosomal localization of porcine prepro-orexin, as a prelude to future candidate gene analysis for feed intake traits.

In order to sequence the gene, a PCR primer pair (forward 5'-AGC GGC AGA CAC CATGAA -3'; reverse 5'-CGA GGT CAG CCC CCC AGA-3') was developed for porcine prepro-orexin based on the published cDNA sequence (GenBank accession no. AF075241.1) from Dyer et al. (1999). The PCR reaction was performed using 12.5 ng of porcine genomic DNA, 1x PCR buffer, 1.25 mM $MgCl_2$, 3.2 mM dNTP, 3 pmol of each primer, 5% dimethyl sulfoxide (DMSO) and .35 U *Taq* DNA polymerase (Promega, Madison, WI) in a 10 μL final volume. The PCR profile included 40 cycles with denaturation at 95-94° C (95° C for 2 min the first cycle, 95 ° C for 1 min the second cycle and 94° C for 1 min the remaining cycles), annealing at 62° C for 40 sec and extension at 72° C for 2 min followed by 30 sec at 4° C. The PCR product was detected with agarose (Metaphor 2%, FMC Bioproducts, ME, USA) gel electrophoresis and ethidium bromide staining.

Five DNA pools (several animals each), representing five breeds (Meishan, Duroc, Hampshire, Landrace, and Yorkshire), were sequenced and sequences examined for polymorphic sites. If found, such sites were used, when possible, to make PCR-RFLPs for genotyping. The PCR product was digested with the restriction enzyme and incubated overnight at 37°C. The digested fragments were separated by 2% Metaphor gel electrophoresis. For linkage mapping all members of the PiGMaP families (Archibald et al. 1995) were then genotyped and two-point linkage analysis was performed using the CRI-MAP program (Green et al. 1990).

For physical gene mapping, a pig/rodent somatic cell hybrid panel developed by Yerle et al. (1996) was used to map this gene in the pig. Primers (forward 5'-ACG CTG CTG CTT CTG CTA CT -3'; and reverse 5'-AGC GGG CAT CCT GAC CAT -3') were used that produced a 251 bp PCR product that was then used for gene mapping. Amplified products were analyzed by 2 % Metaphor gel electrophoresis.

The results for sequencing and mapping are as follows. The 1,247 bp PCR product was confirmed as prepro-orexin as it had a 99% identity to the previously published porcine orexin sequence in a 389 base pair overlap. Our results revealed that the porcine prepro-orexin gene consisted of two exons and one intron distributed over 1,247 bp (Figure 1).

Further sequence analysis revealed three single nucleotide polymorphisms (SNPs), T/C, A/G, and T/C substitutions at position 62 bp, 426 bp, and 974 bp, respectively, in our sequence (GenBank AF169352). The first two SNPs occurred in the intron and the third SNP occurred in exon 2, but did not change the predicted amino acid. The PCR-RFLPs were then redesigned for large scale testing. The first polymorphic site was detected using *BstUI* and a 704 bp fragment was amplified using the primers 5'-AGC GGC AGA CAC CAT GAA TC -3' (forward) and 5'-CAG AGG GCA TTG AGC AAA GGC T -3' (reverse). The second polymorphic site was detected using *AciI* and a fragment was amplified from bases 361 to 686 (325 bp) using the primers 5'-GTA GGT GGA CAA AGC AGC CTG G -3' (forward) and 5'- CAG AGG GCA TTG AGC AAA GGC T -3' (reverse). The PCR-RFLP resulting from the *AciI* digestion of the PCR product produced an undigested PCR product of 325 bp (allele 1) or 65 bp and 260 bp (allele 2) fragments. The third polymorphic site was detected using *NlaIII* and a fragment was amplified from the exon 2 coding region with the primers 5'-ACG CTG CTG CTT CTG CTA CT -3' (forward) and 5'-AGC GGG CAT CCT GAC CAT -

3' (reverse). The *NlaIII* digestion of the PCR product produced a 251 bp fragment (allele 1) and 137 bp and 114 bp polymorphic fragments (allele 2). This site was useful for linkage mapping.

Allele frequencies for the porcine prepro-orexin *NlaIII* PCR-RFLP were determined in 22 grandparental animals from the European PiGMaP families and in 19 unrelated animals from the Iowa State University swine breeding farm. Allele 1 was observed with a frequency of 1.0 in Meishan (n = 9), 0.4 in Hampshire (n=10), 0.167 in Large White (n=20), and 0.0 in Wild Boar (n=2). While these data are interesting, more samples per breed must be collected before any conclusions can be made.

The prepro-orexin gene was physically mapped to SSC12 p13-p11 with complete concordance using the somatic cell hybrid panel (Yerle et al. 1996). The *NlaIII* polymorphic site was genotyped in the PiGMaP reference families (Archibald et al. 1995). Linkage mapping with the PiGMaP families confirmed the physical mapping location of prepro-orexin. The results of two-point analysis showed that the prepro-orexin gene was significantly linked to three markers on porcine chromosome 12 (SSC12). The linked markers were *PRKAR1A* (protein kinase, cAMP-dependent, regulatory, type I, alpha), *GH1* (growth hormone 1) and *BRCA1* (breast cancer 1). The distances from prepro-orexin were 12.5 cM for *PRKAR1A* (Lod=4.7), 9.7 cM for *GH1* (Lod=8.1), and 11.4 cM for *BRCA1* (Lod=5.9). To date, prepro-orexin has not been mapped in the human. Based on information from this mapping study and chromosomal painting (Goureau et al. 1996), we can predict that the human prepro-orexin gene is located on chromosome 17 (q21- q22).

The effect of prepro-orexin on regulating feed intake and the localization of this gene in the pig genome suggest that it may be a candidate gene for appetite. To date, no

quantitative trait loci have been found on chromosome 12 for feed intake and growth (Rothschild and Plastow 1999). Further study on the effect of this gene on feed intake and growth is underway.

Acknowledgments

The authors thank Jeannine Helm for technical support. The authors acknowledge the support of A. Archibald and the EEC for PiGMaP materials and use of the ResPig database. This work is supported in part by PIC USA and PIC Group and the Iowa Agriculture and Home Economics Experimental Station, Ames, Paper no. J- 18568, project no 3148 funds.

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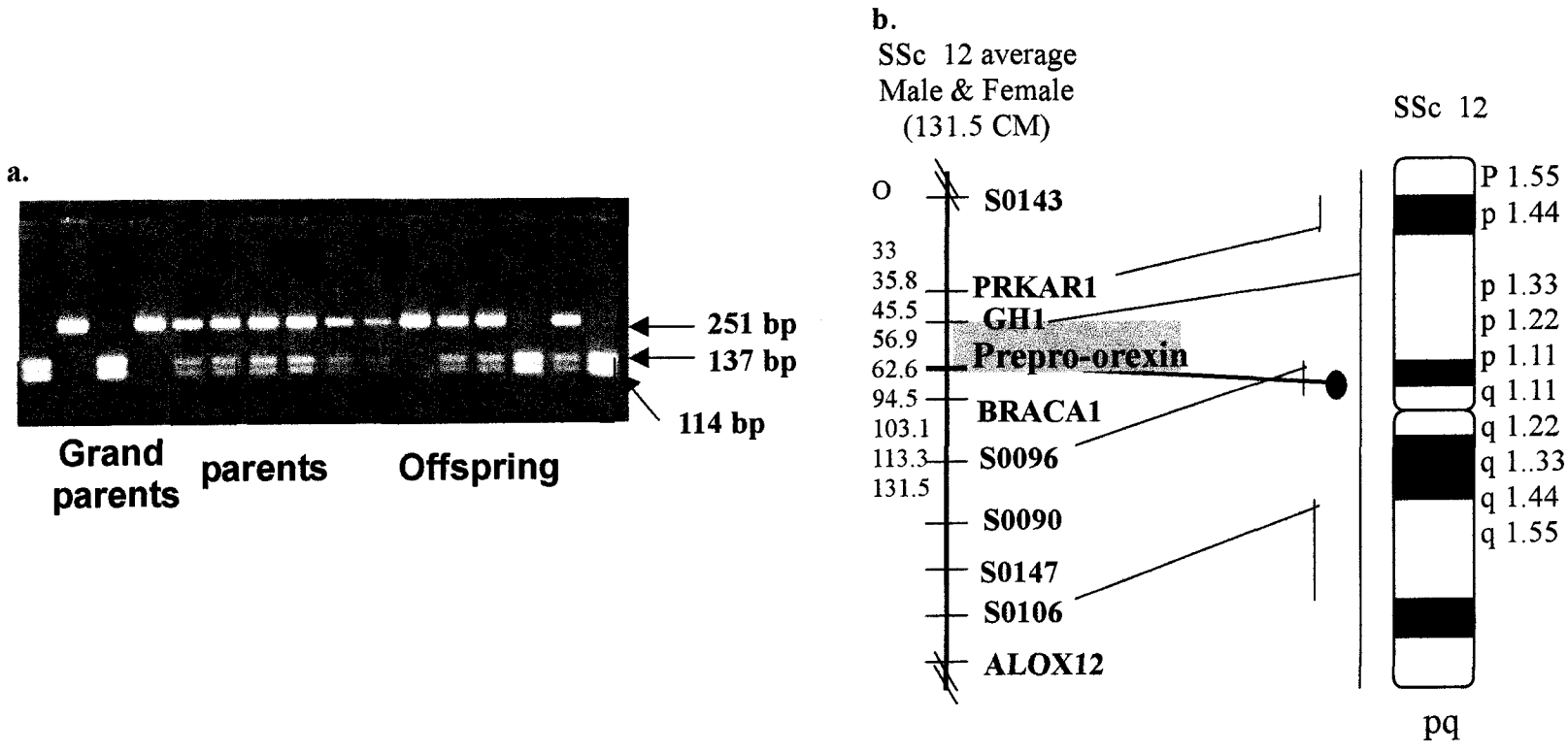
Table1. PCR primers and polymorphic sites in prepro-orexin.

SNP Position [a]		Primer sequences	Size of PCR product [a]	Enzyme	Polymorphic fragments
Pos. 62 T↔C	F:	5'-AGC GGC AGA CAC CAT GAA TC -3'	704 (pos. 1 to 704)	BstU1	Allele 1: 704 bp
	R:	5'-CAG AGG GCA TTG AGC AAA GGC T -3'			Allele 2: 79, 625 bp
Pos. 426 A↔G	F:	5'-GTA GGT GGA CAA AGC AGC CTG G -3'	325 (pos. 361 to 686)	*AciI*	Allele 1: 325 bp
	R:	5'- CAG AGG GCA TTG AGC AAA GGC T -3'			Allele 2: 65, 260 bp
Pos. 974 C↔T	F:	5'-ACG CTG CTG CTT CTG CTA CT –3'	251 (pos. 836 to 1087)	*NlaIII*	Allele 1: 251 bp
	R:	5'-AGC GGG CAT CCT GAC CAT -3'			Allele 2: 137, 114 bp

[a]All numbering is based on GenBank accession numbers AF169352

Figure 1. Gene structure and exon-intron borders for the porcine prepro-orexin gene. The locations of the two exons are indicated by capital letters with gray background, untranslated regions with only capital letters, and the intronic region is in lower case. The location of each primer is underlined and in bold. The polymorphic site is indicated with a larger font in italic format. The upper lines show the sequence of one line and the lower line show the nucleotide difference found in other lines.

Figure 2. a: Family genotyping with *NalIII* PCR-RFLP. Ten F_2 animals are displayed from heterozygous parents. Digestion of the porcine prepro-orexin fragment, analyzed by 2 % Metaphor gel electrophoresis. b: Summary of physical and multi-point linkage maps of the prepro-orexin genes and other markers on SSC12



CHAPTER 7. GENERAL CONCLUSIONS

General Discussion

Pork is an important agricultural commodity in the U.S. with more than 20 billion pounds per year (http://www.usda.gov/nass/aggraphs/lbspr.html) and is a major protein source worldwide. For pork to maintain its position of importance in the human diet, two requirements must be met. First, pork producers must produce pork at a competitive price and this requires a pig with fast and efficient lean growth. Secondly, pork must be low in fat and high in meat quality.

The first objective of this study was to identify chromosomal regions of the pig associated with growth, meat quality and sensory traits based on a molecular genome scan analysis of a three generation Berkshire x Yorkshire cross. Despite the limited differences between the two commercial breeds used in this cross compared with a cross involving an exotic breed, over 100 QTL were detected at the 5% chromosome-wise level for the 40 traits that were evaluated. Significant QTL existed for nearly all traits. Most QTL accounted 3-5% of the phenotypic variance in the F_2 population. Some QTL exceeded this considerably and accounted for nearly 10% of the phenotypic variance. When summed over all QTL, 25 % of the phenotypic variance was explained for some traits. Both breeds had favorable QTL on separate chromosomes for quality traits. In addition, there was some evidence on several chromosomes that cryptic alleles existed that favored the breed least expected to have them. The results of this study demonstrate the feasibility of identifying QTL controlling economically important traits between commercial breeds of pigs. The results also provide researchers with a beginning picture of QTL regions that segregate between the Berkshire

and Yorkshire breeds. The results of the genome scan may allow other researchers to identify the individual genes responsible for the traits of interest. If several of these could be used in marker assisted selection then the improvement could be considerable.

The second objective of this study was to identify and evaluate genes that could be responsible for trait differences for QTL regions identified in the distal region of chromosome 5. These included QTL for meat quality traits (24-hr loin pH, 24-hr loin Minolta, 48-hr loin pH, 48-hr loin Minolta, and 48-hr loin hunter) and body composition (average backfat, last rib backfat, and lumbar backfat), which makes this chromosome important for the study of meat quality and body composition. The estimated position of most of the QTL was at the distal marker (SW378) on this chromosome. Therefore, two additional markers were added distal to SW378 to extend the map coverage for this chromosome. In addition, five genes from human chromosome 12 (*ACACB, PPP1CC, GPR49, DUSP6,* and *ATP2B1*) were chosen as possible candidate genes for meat quality and body composition in this region of SCC5 based on human comparative mapping data and their biological functions. The five genes were successfully mapped in the porcine genome by both physical and genetic methods. Physical mapping results established that the five genes, which are closely linked on human chromosome 12, appear on two different pig chromosomes (5 and 14) due to the presence of a chromosome breakpoint. The mapping of these biologically significant genes in the pig is not only useful for further analysis of QTL associated with meat quality in the pig but may also provide useful information for human biological targets.

Two of the candidate genes, that mapped to the distal region of SSC5 (*DUSP6* and *ATP2B1*) were used for further positional candidate gene analysis. Single nucleotide

polymorphisms (SNPs) were confirmed for each of the candidate genes. Both were silent mutations. The *ATP2B1* gene was located at the center of interval between *SW1954* and *DUSP6*, in which QTL for meat quality were located. The *DUSP6* gene was also located about 10 cM apart from QTL found at distal part of SSC5.

Re-analysis of the QTL regions on SCC5 based on interval mapping, with the addition of the two candidate genes and the two markers, resulted in three additional QTL significant at the 5% chromosome-wise level, for tenth rib backfat, average drip loss, and water holding capacity.

Polymorphisms at the two candidate genes were then used to test for associations of genotype with meat quality and body composition traits. Positional candidate gene association analyses of the BxY F_2 population showed evidence of an association between *ATP2B1* genotypes and body composition (loin eye area and marbling at the tenth rib), growth related traits, glycogen content, and pH related traits. A strong association was observed between *DUSP6* genotypes and fat related traits. This finding confirms the presence of QTL that were detected for these traits on SSC 5. A moderate association of *DUSP6* genotypes with growth related trait was also found. However, in the BxY study we did not find significant QTL for growth related traits for this part of the chromosome. This could be due to limited power to detect QTL for growth related traits.

When both genes were fitted simultaneously, 24-hr loin pH had a weak association with *DUSP6* instead of with *ATP2B1* in the single gene analyses. The inconsistency of these results might suggest that the QTL found for 24-hr loin pH on SSC5 is not caused be the *DUSP6* or *ATP2B1* genes, but rather a closely linked gene. When the interaction between *DUSP6-Pst*I and *ATP2B1-Afl*III genotypes was included, significant interaction effects

($p<0.06$) were found on body composition (last rib backfat and average backfat) and growth (average daily gain to weaning). For these a very interesting interaction was noted. When the *ATP2B1* genotype was 11 or 12, the *DUSP6* 11 genotype was associated with greatest fatness. However, when *ATP2B1* was 22, the fattest pigs had the *DUSP6* 22 genotype, suggesting that these special combinations defined some underlying mutation at another gene.

The B x Y F_2 resource family is a result of a cross between two divergent breeds and as a result extensive linkage disequilibrium is expected. Therefore, it was necessary to investigate and confirm the effects of the *ATP2B1-Afl*III and *DUSP6-Pst*I polymorphisms in closed lines of pigs in order to determine whether these genes are likely to be directly involved in the observed variation in meat quality. Of five commercial lines that were investigated for this purpose, only two were polymorphic for *DUSP6* and one for *ATP2B1*.

The results of the association study for *ATP2B1-Afl*III genotypes in the "C" commercial line did not support the associations that were found in the BxY population for body composition and growth related traits. This difference in results could be due to the line origin of breeds. Therefore, further analysis might be useful to test this gene in other commercial populations. However, the *ATP2B1-Afl*III genotypes had a significant association with light reflectance in the C line, with the 22 genotype having the better color (less light reflectance). A moderate association was also found for *DUSP6-Pst*I genotypes with light reflectance (loin Minolta) and body composition in two commercial lines, with 11 genotype being associated with greatest fatness.

In the commercial line C we tested, we found three haplotypes for the combination of the *ATP2B1* and *DUSP6* genes. Haplotype 2 was the most frequent in line C. The results of

haplotype analysis confirmed that the *ATP2B1* gene is associated with ham Minolta (a and b). The results for backfat thickness (lower backfat) were in line with results of haplotype with the lean meat percentage of the carcass (more body mass) and backfat depth (smaller fat depth). The results of haplotype analysis also showed that the *DUSP6* gene is associated with backfat thickness. There were also some inconsistent results ($p<0.1$) for *DUSP6* (lab Minolta) and *ATP2B1* (probe rib thickness and backfat depth), which can not be explained by the haplotype analysis.

Combined, the QTL analysis and the association studies in the BxY and commercial lines, confirm that fat traits appear to be associated with the *DUSP6* gene. Also the results of this study shows some support for the hypothesis that variation in the *ATP2B1* gene is associated with variation in meat quality. These results also confirmed the expected biological role of each of these candidate genes. However, silent mutations were used in our study to test these associations, therefore further investigation should consider to find the causative polymorphisms.

As discussed in chapter 6, prepro-orexin has been proposed to have a physiological role in the regulation of food intake in the mouse, rat, pig, and human (Mondal et al. 1999). Given the role of *prepro-orexin*, this chapter was designed to characterize the gene structure and chromosomal localization of porcine *prepro-orexin* as a prelude to future candidate gene analyses for feed intake traits. The *prepro-orexin* gene was physically mapped to SSC12. The effect of *prepro-orexin* on regulating feed intake and the localization of this gene in the pig genome suggest that it may be a candidate gene for appetite. To date, no quantitative trait loci have been found on chromosome 12 for feed intake and growth.

Recommendations for Future Research

Several QTL regions with possible effects on meat quality were identified in a cross between two commercial breeds (BxY). In this study only single QTL models were considered with the aim to detect QTL that were segregating between the Berkshire and Yorkshire breeds. Additional statistical methods must be considered for analyzing the QTL data, including multiple QTL models. Epistasis among QTL and imprinted QTL may also be important. With the current model we were not able to distinguish these effects, therefore it will be useful to use an improved model, which takes these effects into account.

We were able to further investigate QTL in a region of the q-arm of SSC5. Therefore further study is needed to identify and investigate candidate genes for other regions of the swine genome for growth, body composition and meat quality.

Breeding organizations want to apply QTL results in their breeding schemes. However, confirmation studies of the putative QTL detected in this study will be required before the information is implemented. Also, a joint analysis that combines data from populations with similar crosses will be required to detect QTL which was not found in this study.

The *ATP2B1-Af*lIII and *DUSP6-Af*lIIIpolymorphisms were informative in only one and two commercial lines, respectively. Therefore more informative polymorphisms in both genes must be identified, in order to further test associations in other commercial lines.

Due to some inconsistent results in some of association studies, further analyses are needed before drawing final conclusions on candidate gene analysis. Also in our study we were not able to confirm if the polymorphisms for the candidate genes that were investigated are the causative polymorphisms for the observed effects. Improved methods such as positional cloning would allow us to confirm whether these genes have a causative effect.

APPENDIX A. CORRELATIONS AMONG SELECTED PORK QUALITY TRAITS[1]

A paper accepted by the Journal of Animal Science

E. Huff-Lonergan[2], T.J. Baas[2], M. Malek[2], J.C.M. Dekkers[2], K. Prusa[3], and M.F. Rothschild[2]

Abstract

Mechanisms underlying the development of pork quality traits are elusive. Establishing relationships among specific quality traits is important if significant progress toward developing improved meat quality is to be realized. As part of a study to examine the individual effects of genes on meat quality traits in pigs, a three-generation resource family was developed. Two Berkshire sires and nine Yorkshire dams were used to produce nine F1 litters. Sixty-five matings were made from the F1 litters to produce four sets of F2 offspring for a total of 525 F2 animals that were used in the study. These F2 animals were slaughtered at a commercial facility upon reaching approximately 110 kg. Carcass composition traits, pH measurements, and subjective quality scores were made at 24 h postmortem (PM). Loin samples (n = 525) were collected at 48 h PM, and meat quality traits were evaluated. These traits included pH (48 h), Hunter L values, drip loss, glycolytic potential, ratio of type IIa/IIb myosin heavy chains (IIa/IIb), total lipid, instrumental measures of tenderness using the Star Probe attachment of the Instron, cook loss measurements, and sensory evaluations.

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Significant correlations were found between many carcass, instrumental and biochemical measurements, and sensory quality traits. Star Probe measurements were significantly correlated to drip loss (0.29), glycolytic potential (0.30), pH (-0.29), total lipid (-0.14), and Hunter L values (0.28). Drip loss was significantly correlated to glycolytic potential (0.36), pH (-0.28), IIa/IIb (-0.10), and Hunter L values (0.33). Hunter L values were also significantly correlated to total lipid (0.33) and IIa/IIb (-0.11). Sensory tenderness, flavor and off-flavor scores were significantly correlated to drip loss, pH and glycolytic potential measurements. Marbling score, total lipid, and drip loss were not significantly correlated to sensory juiciness scores, but cooking loss was. Marbling and total lipid were significantly correlated to firmness scores (0.37 and 0.31, respectively). Taken together, the data in this study suggest that changes in some meat quality traits can affect many other meat quality attributes. The correlations reported in this study yield important information that can be used to aid in directing future studies aimed at understanding the underlying biological mechanisms behind the development of many quality traits.

Key words: Pork Quality, pH, Tenderness, Water-Holding Capacity, Color, Glycolytic Potential

Introduction

Evaluation of pork quality traits is of particular importance if improvement of these traits are to be realized. For many years, one of the major objectives of the swine industry was to increase the lean to fat ratio of pork carcasses (Cameron, 1990; Cliplef and McKay, 1993). As a result, dramatic improvements in the body composition of pigs have been made through

genetic selection (Sellier and Rothschild, 1991). More recently, an increasing amount of emphasis has been placed on improving pork quality traits (Hovenier et al., 1993a). Improvements in these characteristics have proven to be somewhat more elusive. Pork quality traits are often truly composite traits, being influenced by several antemortem and postmortem factors (Gregory, 1987; Honikel, 1987; Warriss, 1987; Sellier and Monin, 1994), thereby making the prediction of ultimate pork quality more difficult. In turn, this fact makes genetic selection for improved pork quality more difficult as well (deVries and van derWal. 1993). Many physical and biochemical factors have been evaluated in an attempt to assess meat quality. Some of these include Hunter L values (measures of the relative lightness or darkness of the product), marbling and lipid content, ultimate pH of the meat, glycolytic potential (estimate of the muscle glycogen content at slaughter) and muscle fiber type (Honikel, 1993; Sellier and Monin, 1994). However, a limited number of studies have made a comprehensive effort to look at a wide variety of biochemical, sensory and physical traits in one population. To be able to begin to control variation in pork quality, it is important to understand the relationships between biochemical measurements with sensory and processing characteristics. The objective of this study was to determine phenotypic associations between specific biochemical and physical parameters and sensory characteristics in order to obtain a better understanding of how changes in specific traits may influence pork quality overall.

Materials and Methods

Animals

Data from a three-generation resource family that was established to map genes that affect meat quality was used. To establish this group of pigs, two purebred Berkshire boars and

nine purebred Yorkshire females were used to produce nine F1 litters. The boars were chosen from commercial boar studs and were mated artificially to sows from the Iowa State University Swine Breeding Farm. From the resulting F1 litters, 8 boars and 26 gilts were selected to produce the 525 F2 animals used in this study. A total of 65 matings was made to produce four sets of F2 offspring. The pigs were weighed at weekly intervals and sent to a commercial facility to be slaughtered when they reached approximately 110 kg. Details of the rearing and management procedures are described in Malek et al. (2001).

After slaughter and chilling, carcass traits were evaluated by trained university personnel at the plant according to the National Pork Producers Council guidelines (NPPC, 1991). These data included carcass composition traits, 24-hour pH measured with a glass penetration pH electrode, and the subjective quality traits of marbling, firmness, and color in the loin (on 1-5 scale). Loins were removed from the carcasses and transported back to the Iowa State University Meat Lab. At 48 hours postmortem, pH was again measured with a glass penetration pH electrode, Hunter L values were collected, and loin samples were collected for further evaluation.

Laboratory Evaluations

Drip loss was measured on a size-standardized sample from the longissimus dorsi (3 cm in diameter x 2.5 cm thick) (Honikel et al., 1986; Kauffman et al., 1986) that was collected at 48 hours postmortem. The sample was weighed, suspended in a plastic bag, and held at 4°C for 72 hours. The sample was re-weighed at the end of the holding time. Drip loss was calculated as the percentage of product weight that was lost over 72 hours of storage.

At 48 hours postmortem, a sub-sample of the loin was frozen and sent to the University of Illinois where glycogen, free glucose, glucose-6-P, and lactate were measured. Glycolytic potential was calculated as follows: glycolytic potential = 2 x ([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate]. (Monin and Sellier, 1985; Maribo et al., 1999). The values for glycolytic potential were expressed as μM lactate equivalents per gram muscle wet weight. The values for total glycogen (μM/g) and lactate (μM/g) as well as glycolytic potential were used in the study.

Total lipid in the longissimus dorsi was measured following the method of Bligh and Dyer (1959) and expressed as a percentage of the tissue. Total lipids were dissolved in isopropanol and assayed for concentration of total cholesterol using an enzymatic procedure (Sigma Cholesterol Kit No. 352, Sigma Chemical Co., St. Louis, MO). Cholesterol was reported as mg/100 g of tissue.

Differences in muscle fiber type were evaluated in 48-hour postmortem samples from the longissimus dorsi by separation of myosin isoforms on high porosity SDS-PAGE gels. The procedure used was as described by Talmadge and Roy (1993) with modifications to further improve myosin isoform separation. Muscle samples were extracted in 9 volumes of ice-cold homogenization buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA, and 20 mM Tris, pH 6.8) using a Dounce homogenizer and centrifuged at 1500 x g for 15 minutes at 4°C. The pellets were homogenized with a Dounce homogenizer in 25 volumes of whole muscle extraction buffer (2% sodium dodecyl sulfate (SDS) (wt/vol), 10 mM sodium phosphate, pH 7.0). Samples were then centrifuged at 1500 x g for 15 minutes at 20°C. (Huff-Lonergan et al., 1996). Protein concentration of the supernatant was determined using the BioRad DC assay (modification of Lowry et al., 1951). Samples were heated in sample buffer (Laemmli,

1970) at 50°C for 20 minutes at a final protein concentration of .125 mg/ml, and a total of 2 μg of protein was loaded on each lane of 18 cm x 16 cm x 1.5 mm SDS-PAGE gels. The stacking gels were composed of 30% glycerol, 4% acrylamide-N,N'-methylene-bis-acrylamide (acrylamide-bis) (50:1), 70 mM Tris (pH 6.7), 4 mM EDTA, and .4% SDS. The separating gels were composed of 30% glycerol, 6% acrylamide-bis (50:1), 200 mM Tris (pH 8.8), 100 mM glycine, and .4% SDS. Polymerization of stacking and separating gels was initiated with .05% N,N,N',N'-tetramethylethylenediamine (TEMED) and .1% ammonium persulfate. The upper running buffer consisted of 100 mM Tris (base), 150 mM glycine, .1% β-mercaptoethanol, and .1% SDS. The lower running buffer consisted of 50 mM Tris (base), 75 mM glycine, and 1% SDS. Gels and running buffers were cooled to 4°C before use. Gels were run at 250 V for 24 hours at 4°C. Bands were visualized by staining with Coomassie Blue. Gel images were captured using a Kodak DC120 digital camera and the images were analyzed using the Kodak 1D Image Analysis software. Results were expressed as the ratio of the density of the IIa band of myosin to the density of the IIb band within a sample. Porcine diaphragm muscle (extracted as outlined above) was used as a standard on each gel to aid in identifying the isoforms of myosin. Diaphragm muscle contains primarily type IIa, IIx, and type I associated myosin isoforms (Talmadge and Roy, 1993).

For evaluation of the cooked product, vacuum-packaged boneless chops from the longissimus dorsi of each animal were stored for 10 days at 4°C. Following the storage period, chops were broiled to 71°C under an electric oven broiler (Amana Model ARE 60) that had been previously preheated to 210°C. The temperature of each chop was monitored using thermocouples (Chromega/Alomega) attached to an Omega digital thermometer (Model DSS-650, Omega Engineering. Instrumental measurement of tenderness was

evaluated using a circular, five-pointed star-probe (9 mm in diameter with 6 mm between each point) attached to an Instron Universal Testing Machine (Model 1122). A 100 kg load cell was used with a crosshead speed of 200 mm/min. The star-probe attachment was used to determine the amount of force needed to puncture and compress the chop to 80% of the sample height. Each chop was punctured 3 times and an average was recorded. Sensory evaluation was done using a highly-trained professional sensory panel. Cubes, 1.3-cm in size, were removed from the center of the broiled loin chops. The cubes from each loin were placed in preheated, individually-coded glass petri dishes and served to each panelist. Samples were evaluated for degree of juiciness, tenderness, chewiness, pork flavor, and off-flavor using a 10-point category scale. The scale was anchored on the left end with a term representing a low degree of juiciness, tenderness, chewiness, flavor, and off-flavor intensity. On the right end of the scale was a term representing a high degree of each characteristic. Each panelist was seated at an individual booth with red lighting overhead. Room temperature deionized, distilled water and unsalted crackers were used to cleanse the palate of the panelists between samples.

Statistical Analysis

In order to obtain and compare values observed in this study with those from previous studies means and standard deviations were calculated for all traits. Associations between traits (phenotypic) were determined by calculating phenotypic correlations using the computer package developed by SAS (SAS/STAT, 1990). Genetic correlations were not calculated due to the small numbers of sires used in this study.

Results and Discussion

The means for the major traits evaluated in this population are presented in Table 1. In general, the meat quality characteristics for this F2 population were relatively high and compared favorably with the results obtained in the NPPC National Genetic Evaluation Program (Johnson and Goodwin, 1995) for the two purebred foundation breeds (Berkshire and Yorkshire) used to generate the F2 population used in this study. These breeds were chosen for their divergence in meat quality characteristics. Standard deviations of the traits were also similar to those expected. Few studies have been published on the Berkshire breed and these results with (on average) 50% Berkshire genetics should be of interest given the recent interest in the Berkshire breed in the United States.

Correlations Among Important Pork Quality Traits

There were significant correlations among many of the quality traits that are important for both fresh pork sensory attributes and processing factors (Table 2). Subjective color, marbling, and firmness scores were all significantly positively correlated with most sensory traits except juiciness. Subjective color was also significantly correlated with firmness, drip loss, and instrumental evaluation of tenderness (Star Probe). These data indicate that in this study, darker product had a greater propensity to be firmer, have less drip loss and be more tender. While the correlation between subjective color and flavor was significant, the magnitude of the correlation is small enough to indicate that in this population, other factors may have a larger influence on flavor.

Marbling was significantly correlated with firmness, drip loss, percent cook loss, and measures of tenderness (Table 2). Of these traits, the strongest correlation was between

marbling and firmness. The positive correlation observed indicated that product with a higher degree of marbling also tended to be evaluated as firmer. The same relationship was found when comparing percent intramuscular lipid and product firmness (Table 3). It is possible that in chilled product higher amounts of lipid may aid in improving the firmness of the product. The modest relationship between marbling and/or lipid and instrumental measurements of tenderness is in agreement with several studies showing that higher levels of marbling are related to lower shear force (De Vol., et al., 1988; Ramsey et al., 1990; Hodgson et al., 1991; Candek-Potokar et al., 1998). However, numerous other authors have shown little or no consistent relationship of measures of marbling or lipid with measures of tenderness (DeVol et al., 1988; Hovenier et al., 1993b; Tornberg et al., 1993; Jones et al., 1994; Blanchard et al., 2000). Other parameters such as proteolysis and myofibrillar fragmentation may also play a role in determining tenderness (Wheeler et al., 2000).

Interestingly, in this study, neither marbling (Table 2) nor percent intramuscular lipid (Table 3) was significantly correlated with juiciness score. Other investigators have also found this relationship (Cameron, 1990; Blanchard et al., 2000). However, both marbling and percent intramuscular lipid were positively correlated with flavor scores and negatively correlated with off-flavor scores, indicating that the panelists in this study associated more normal pork flavor with products that had higher marbling and/or percent intramuscular lipid. It is important to note, however, that the magnitude of these correlations was not extremely high, therefore, other factors also influence the perception of pork flavor.

Drip loss has long been primarily evaluated as a key parameter in processing situations when product yield is important. It is apparent in this study that drip loss may be related to sensory parameters of fresh product as well; drip loss was significantly correlated

to measures of subjective color, tenderness (Star Probe and tenderness score), and flavor of fresh product. These data indicate that product with a high degree of drip loss would also tend to be lighter in color, less tender, have less pork flavor, and more off-flavor.

When considering the relationships between the sensory traits of tenderness, juiciness, flavor, and off-flavor, many significant correlations were observed (Table 2). Tenderness was most highly correlated with juiciness, flavor, and off-flavor, in a manner that indicated that panelists in this study tended to rate more tender products as also being more juicy, having more pork flavor, and having less off-flavor. In this study, product that lost more weight during cooking had a tendency to be rated as being less juicy. This is as expected, because a large proportion of the weight lost during cooking results from moisture loss. It is interesting to note that drip loss of the product (moisture lost prior to cooking) was not significantly correlated with juiciness and that the correlation between cook loss and drip loss, while significant, was low (0.16, Table 2). This is not surprising, as moisture lost prior to cooking obviously could not be lost during cooking.

This study also provided a venue to compare the instrumental measure of tenderness (Star Probe) with sensory evaluations of tenderness. The Star Probe device measures the amount of force needed to both puncture and compress a meat sample, a procedure similar to what takes place in the first chews by human molars. The relationships between sensory tenderness measurements and Star Probe measurements were significant and were moderate in magnitude (Table 2), indicating relatively good agreement between the measures. The negative correlation indicates that product that required less force to puncture and compress it would also tend to be rated as being more tender.

Correlations Among Pork Quality Traits and Biochemical / Instrumental Predictors of Quality.

Because in many cases it is not practical to measure sensory, textural, and processing traits directly and because many of these traits are subjective in nature, it is important to know the relationship between these traits and many of the commonly used objective instrument-based measures of pork quality. Also, in the search for genetic markers to aid in producing animals that consistently produce high quality products, it is important to know the biochemical mechanisms behind the development of quality traits. The correlations that are reported in Table 3 begin to provide more insight into some of these mechanisms.

Carcass weight was significantly correlated only with marbling, juiciness score, and off-flavor score. However, the magnitude of these correlations was relatively small, indicating that carcass weight by itself may not have a large influence on pork quality. The carcass measurements of 10^{th} rib backfat and loin eye area were significantly correlated with several quality traits. Tenth rib backfat was significantly correlated with marbling, firmness, tenderness (Star Probe and sensory tenderness), flavor, and off-flavor. The correlation between 10^{th} rib backfat and color was also significant but the magnitude of this correlation was relatively low. Loin eye area was significantly correlated with marbling, tenderness (Star Probe and sensory tenderness), flavor, and off-flavor scores. These correlations suggest that there could be a tendency for leaner, heavier muscled carcasses in this study to have less marbling in the loin, and to have loin chops that are less firm, less tender, and that have less characteristic pork flavor than chops from carcasses with greater amounts of 10^{th} rib backfat and/or smaller loin eyes.

pH and Glycolytic Potential. Post-rigor pH measurements are common measurements that are used to predict several meat quality traits. To gain a better understanding of the mechanism behind the variation in postmortem pH it is important to examine relationships between pork quality measures and some of the biochemical parameters related to muscle pH. Therefore, factors relating to postmortem glycolysis were also evaluated.

Loin pH measurements were taken both at 24 hours and at 48 hours postmortem. The correlation between these two measurements was significant, and the magnitude of the correlation was relatively high (0.71, Table 4). Correlations of pH measured at 24 or 48 hours postmortem with other quality measurements were very similar (Table 3). Significant correlations were found between both 24 hour and 48 hour pH measurements and many of the quality traits measured. Traits that were most highly correlated with pH (both 24 h and 48h) measures were; color, drip loss, tenderness (Star Probe and sensory tenderness), flavor and off-flavor scores, and cook loss. These results indicate that a lower ultimate pH of the product was associated with lighter colored product, product with higher drip loss, less tender product, and with less pork flavor and more off-flavor in the product.

Glycolytic potential is an estimate of the amount of glycogen that is present in the muscle at slaughter. Greater amounts of glycogen in the tissue at slaughter can provide the potential for a sustained glycolysis in the muscle after slaughter, which could result in lower ultimate pH. Indeed, very high glycolytic potential values are associated with significantly lower ultimate pH values observed in meat from pigs with the RN gene (Monin and Sellier, 1985). In this study, glycolytic potential had a significant positive correlation with Hunter L values and drip loss and was significantly negatively correlated with pH (Table 4). Lower glycolytic potential was associated with product that was more tender, juicier, and that was

evaluated as having more pork flavor and less off-flavor. Similar relationships were also seen for lactate (Table 3). Residual glycogen was also significantly correlated with measures of pH (Table 4), tenderness, and pork flavor evaluations (Table 3), but unlike glycolytic potential and lactate content, residual glycogen was not significantly correlated with juiciness or off-flavor scores.

Color. Subjective color of the product was significantly correlated with Hunter L values, an instrumental measure of the lightness/darkness of the product. The magnitude of this correlation was relatively high (-0.69, Table 3), indicating that Hunter L values are predictive of subjective color scores. One reason that the magnitude of the correlation was not higher could be that subjective scores of color often take into account not only the lightness/darkness of the product, but also the amount of redness observed in the product. Nevertheless, both subjective color and Hunter L values were significantly correlated to most of the same traits. The post-rigor pH measures of the product (both 24 and 48-hour) were among the traits measured that had the highest correlations to both subjective color score (Table 3) and Hunter L values (Table 4). Additionally, traits that are closely related to ultimate pH (glycolytic potential and lactate content) were also among the traits that were significantly correlated with color measures (Tables 3 and 4).

In this study, products that were evaluated as having a higher color score or a lower Hunter L value (darker) tended to be associated with a higher post-rigor pH. This relationship is not uncommon (DeVol et al., 1988; Hovenier et al., 1992). Pork with high ultimate pH often is darker in color. Conversely, pork with a low ultimate pH can be lighter in color (Monin and Sellier, 1985). One reason for this is that low pH values may result in denaturation of myoglobin and other muscle proteins, which can cause the meat to appear

lighter in color. Denaturation of proteins reduces their solubility and causes them to precipitate and to reflect rather than absorb light, resulting in lighter colored product (Honikel, 1987).

Firmness. The biochemical/instrumental measures that were most closely correlated with firmness were lipid content, color measures (subjective color, Table 2, Hunter L, Table 3), post-rigor pH measures, lactate content, and glycolytic potential (Table 3). However, post-rigor glycogen content was not significantly correlated with firmness (Table 3). Post-rigor pH is often associated with firmness of the product, with product having a high ultimate pH often categorized as being firmer. In general, it appears that these same relationships held in the population used in this study.

Percent Drip Loss and Percent Cook Loss. Biochemical and instrumental traits that were most closely associated with drip loss and percent cook loss included Hunter L values, post-rigor pH, and factors related to the development of ultimate pH (glycolytic potential, residual glycogen and lactate content) (Table 3). These relationships indicated that product with a low ultimate pH (and high lactate content) and a high glycolytic potential might be expected to have higher drip loss. Similar phenotypic relationships have been reported in other populations (Hovenier et al., 1992; Hovenier et al., 1993b; de Vries et al., 1994; Lonergan et al., 2001).

Palatability Traits. Evaluations of tenderness (Star Probe measurements and sensory panel tenderness scores), juiciness, flavor, and off-flavor were most highly correlated to ultimate pH measures and to biochemical factors that are expected to impact the extent of pH decline (residual glycogen, lactate and glycolytic potential) (Table 3). Relationships between tenderness and pH were also noted by DeVol et al. (1988) and Cameron (1990). These data

indicate that higher ultimate pH tends to be associated with more desirable eating qualities (more tender and juicy, greater pork flavor, and less off-flavor).

Muscle Fiber Characteristics. Muscle fibers can be classified by metabolic characteristics as type I (slow twitch, oxidative), type IIa (fast twitch, oxidative-glycolytic), and type IIb (fast twitch, glycolytic). Type IIb fibers tend to have less myoglobin and be larger in diameter than other fiber types. Some studies have indicated that selection for rapid growth rate or less backfat could alter muscle fiber composition toward a higher percentage of type IIb fibers (Rahelic and Puac, 1981; Brocks et al., 2000). Ratios of these fiber types may influence metabolic properties of the muscle. In turn, this could result in changes in muscle metabolism after slaughter (Essen-Gustavsson, 1993). Muscle fibers that are more reliant on glycolytic pathways and that contain less myoglobin (IIb) to store oxygen may shift to anaerobic metabolism earlier, thereby accelerating the rate of postmortem pH decline. It could be hypothesized that a lower IIa/IIb ratio (higher proportion of type IIb fibers) would result in lighter colored product and more rapid pH decline.

In the current study, there was a significant correlation of the relative proportion of type IIa to type IIb fibers with the quality characteristics of color (subjective color, Table 3, and Hunter L, Table 4), firmness, and drip loss (Table 3), indicating that products with a higher percentage of IIb fibers could have a greater propensity to be lighter in color, less firm and have greater drip loss. Product that is less firm, and that has a higher drip loss is often associated with more rapid pH decline. While there was not a significant correlation between the ratio of IIa/IIb fibers and ultimate pH (Table 5), pre-rigor pH decline was not measured, so the relationship between early pH decline and fiber type cannot be determined. There was also a significant correlation of the ratio of type IIa to type IIb with the carcass characteristics

of 10^{th} rib backfat and loin eye area. These data suggest that there could be a tendency for carcasses with a greater amount of type IIb fibers in the loin to have less 10^{th} rib backfat and to have slightly larger loin eyes and for the product to be lighter in color, less firm, and have higher drip loss. However, the magnitude of these relationships was not high, indicating that other factors also contribute to these characteristics. Larzul et al. (1997) found very similar phenotypic corrleations of the percentage of type IIb fibers with average backfat (0.14) and L* values(0.18).

Relationships Among Carcass Measurements and Chemical Characteristics. Carcass weight was significantly positively correlated to 10^{th} rib backfat, loin eye area, residual glycogen, lactate content, and glycolytic potential (Table 4). Tenth rib backfat was most highly correlated with loin eye area (-0.57) and percent lipid in the loin (0.45). Loin eye area was also significantly correlated with percent lipid in the loin (-0.27). These data indicated that selection for less backfat might result in carcasses with larger loin eyes and a lower percentage lipid in the loin.

Percent lipid in the loin was positively and moderately correlated with Hunter L values, such that product that had a higher percentage of lipid could be expected to have a higher Hunter L value (lighter colored product) (Table 4).

Correlations Between Muscles. Often it is of interest to know whether pH or color measurements made in the longissimus have the potential to be predictive of the pH or color of the ham, in this case the major ham muscle, the semimembranosus. In this population of pigs, the pH of the semimembranosus was significantly correlated to ultimate pH measures in the loin (Table 5). The magnitude of the correlation was moderate indicating some predictive value. Hunter L values measured at 24 h postmortem in the semimembranosus were also

significantly correlated to the Hunter L values in the loin at 24 and 48 h postmortem (Table 5). Again, the magnitude of the correlation was only moderate, indicating moderate reliability in predicting the color of one muscle based on the color of the another.

Implications

Meat quality traits are very complex and are influenced by many factors. This fact makes the prediction of these traits very difficult, especially when attempting to develop improvement strategies for pork quality. Understanding the relationships between numerous quality parameters is therefore important if progress is to be made. This study is unique in the scope of the quality traits that were examined. There were significant correlations between many quality traits that are important for both palatability of the fresh product and for processing factors. These data suggest that changes in some meat quality traits can affect many other meat quality attributes. In addition, there were many significant correlations between biochemical traits, instrumental measures of quality, and sensory characteristics. Therefore, the correlations reported in this study yield important information that can be used to aid in directing future studies aimed at elucidating the underlying biological mechanisms behind the development of many quality traits.

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Table 1. Means and standard deviations of the traits measured.

Trait	n	Mean	Standard Deviation
Carcass Characteristics			
Carcass Weight	525	87.08 kg	5.73
10^{th} rib back fat	525	3.19 cm	.779
Last rib back fat	525	3.16 cm	.609
Loin eye area	525	35.59 cm^2	5.684
Meat Quality Characteristics			
Subjective Color (Loin) (1-5 scale)	525	3.25	.482
Subjective Marbling (Loin) (1-5 scale)	525	3.80	.732
Subjective Firmness (Loin) (1-5 scale)	525	3.42	.627
24 hour semimembranosus pH	525	5.90	.218
24 hour loin pH	525	5.78	.174
48 hour loin pH	525	5.83	.190
Loin Hunter L (24 hours)	524	44.07	6.12
Loin Hunter L (48 hours)	525	46.87	3.39
Semimembranosus Hunter L (24 hours)	525	41.65	3.46
Drip Loss (Loin)	525	5.84%	1.99
Cook Loss (Loin)	514	18.23%	4.40
Biochemical Measures			
Glycogen	519	8.68 μM/g	3.34
Lactate	519	86.67 μM/g	13.30
Glycolytic Potential	518	104.00 μM/g	16.31
Ratio IIa/IIb fiber type	513	1.04	.77
Total Lipid	525	3.23%	1.318
Total Cholesterol	525	57.72 mg/100 g	8.29
Textural and Sensory Characteristics			
Star Probe Penetration Force	488	4.36 kg	.863
Sensory Tenderness Score (1-10 scale)	513	7.84	1.17
Sensory Juiciness Score (1-10 scale)	513	6.02	1.49
Sensory Flavor Score (1-10 scale)	513	2.85	1.76
Sensory Off-Flavor Score (1-10 scale)	513	1.59	2.03

Table 2. Correlations between subjective evaluations, drip loss and cooking loss, instrumental measurement of tenderness and sensory characteristics of the longissimus dorsi.

	Color (loin)	Marbling (loin)	Firmness (loin)	Drip Loss (loin)	% Cook Loss	Star Probe (loin)	Tender. Score (loin)	Juiciness Score (loin)	Flavor Score (loin)
Marbling	.03 (.4972)								
Firmness	.27 (.0001)	.37 (.0001)							
Drip loss (loin)	-.33 (.0001)	-.12 (.0049)	-.25 (.0001)						
% Cook Loss	-.21 (.0001)	-.11 (.0131)	-.12 (.0075)	.16 (.0004)					
Star Probe	-.27 (.0001)	-.27 (.0001)	-.21 (.0001)	.29 (.0001)	.34 (.0001)				
Tender. Score	.19 (.0001)	.21 (.0001)	.21 (.0001)	-.30 (.0001)	-.28 (.0001)	-.54 (.0001)			
Juiciness Score	.07 (.14)	.02 (.59)	.07 (.09)	-.05 (.23)	-.43 (.0001)	-.16 (.0005)	.46 (.0001)		
Flavor Score	.09 (.05)	.20 (.0001)	.16 (.0002)	-.24 (.0001)	.12 (.0054)	-.21 (.0001)	.37 (.0001)	.12 (.01)	
Off-Flavor Score	-.17 (.0001)	-.15 (.0005)	-.18 (.0001)	.35 (.0001)	-.03 (.5352)	.13 (.004)	-.30 (.0001)	-.08 (.06)	-.62 (.0001)

Upper row = phenotypic correlations
P values for difference from zero in parenthesis

Table 3. Correlations between pork carcass measurements and biochemical measurements of the longissimus dorsi and sensory characteristics.

	Carcass Weight	10^{th} rib BF	Loin Eye Area	pH 24 hr (loin)	pH 48 hr (loin)	Loin Hunter L (48 hr)	IIa/IIb (loin)	Glycogen (loin)	Lactate (loin)	Glycolytic Potential (loin)	Lipid (loin)	Cholest. (loin)
Color	.06 (.18)	-.10 (.02)	.07 (.13)	.30 (.003)	.28 (.0001)	-.69 (.0001)	.13 (.003)	-.18 (.0001)	-.27 (.0001)	-.30 (.0001)	-.15 (.0001)	-.07 (.03)
Marbling	.09 (.04)	.38 (.0001)	-.25 (.0001)	.13 (.003)	.15 (.0006)	.04 (.35)	.06 (.18)	-.08 (.06)	-.10 (.02)	-.12 (.01)	.57 (.0001)	.09 (.04)
Firmness	.08 (.06)	.24 (.0001)	-.11 (.01)	.20 (.0001)	.21 (.0001)	-.20 (.0001)	.11 (.01)	-.08 (.07)	-.23 (.0001)	-.22 (.0001)	.31 (.0001)	.02 (.72)
Drip loss	.01 (.83)	.01 (.88)	.02 (.64)	-.33 (.0001)	-.28 (.0001)	.33 (.0001)	-.10 (.03)	.21 (.0001)	.34 (.0001)	.36 (.0001)	-.01 (.83)	.00 (.97)
% Cook Loss	-.03 (.4853)	.11 (.0154)	-.06 (.1853)	-.20 (.0001)	-.20 (.0001)	.31 (.0001)	.02 (.5776)	.21 (.0001)	.19 (.0001)	.24 (.0001)	.12 (.006)	.07 (.0989)
Star Probe	-.01 (.74)	-.19 (.0001)	.22 (.0001)	-.31 (.0001)	-.29 (.0001)	.28 (.0001)	-.08 (.08)	.25 (.0001)	.24 (.0001)	.30 (.0001)	-.14 (.002)	.08 (.10)
Tender. Score	.03 (.57)	.19 (.0001)	-.18 (.0001)	.27 (.0001)	.28 (.0001)	-.15 (.0004)	.02 (.59)	-.20 (.0001)	-.28 (.0001)	-.31 (.0001)	.19 (.0001)	-.12 (.01)
Juiciness Score	.09 (.04)	.01 (.83)	.07 (.09)	.17 (.0001)	.15 (.001)	-.02 (.64)	-.06 (.18)	-.07 (.11)	-.22 (.0001)	-.21 (.0001)	.05 (.27)	-.09 (.04)
Flavor Score	.05 (.27)	.24 (.0001)	-.16 (.0001)	.25 (.0001)	.32 (.0001)	-.04 (.38)	-.01 (.86)	-.13 (.003)	-.23 (.0001)	-.24 (.0001)	.23 (.0001)	-.04 (.41)
Off-Flavor Score	.14 (.002)	-.21 (.0001)	.10 (.03)	-.23 (.0001)	-.32 (.0001)	.12 (.01)	.01 (.86)	.07 (.14)	.20 (.0001)	.19 (.0001)	-.19 (.0001)	-.02 (.60)

Upper row = phenotypic correlations
P values for difference from zero in parenthesis

Table 4. Correlations between pork carcass measurements and biochemical measurements of the longissimus dorsi.

	Carcass weight	10^{th} rib BF	LEA	pH 24 hr (loin)	pH 48 hr (loin)	Loin Hunter L (48 r)	IIa/IIb (loin)	Glycogen (loin)	Lactate (loin)	Glycolytic Potential (loin)	Lipid (loin)
10^{th} rib BF	.27 (.0001)										
LEA	.17 (.0001)	-.57 (.0001)									
pH 24 hr (loin)	-.03 (.46)	.02 (.68)	-.01 (.80)								
pH 48 hr (loin)	-.03 (.51)	.02 (.60)	-.04 (.41)	.71 (.0001)							
Loin Hunter L (48 hr)	-.03 (.46)	.19 (.0001)	-.13 (.002)	-.32 (.0001)	-.27 (.0001)						
IIa/IIb (loin)	-.00 (.94)	.14 (.002)	-.13 (.004)	.02 (.64)	-.01 (.77)	-.11 (.02)					
Glycogen (loin)	.10 (.02)	.08 (.08)	.10 (.02)	-.21 (.0001)	-.15 (.0007)	.22 (.0001)	-.02 (.70)				
Lactate (loin)	.10 (.02)	.08 (.07)	-.06 (.19)	-.37 (.0001)	-.38 (.0001)	.26 (.0001)	-.02 (.72)	.25 (.0001)			
Glycolytic Potential (loin)	.12 (.006)	.10 (.03)	-.01 (.86)	-.38 (.0001)	-.39 (.0001)	.30 (.0001)	-.02 (.67)	.61 (.0001)	.92 (.0001)		
Lipid (loin)	.03 (.54)	.45 (.0001)	-.27 (.0001)	.02 (.57)	.08 (.07)	.33 (.0001)	.04 (.36)	.06 (.19)	-.04 (.32)	-.01 (.79)	
Cholesterol (loin)	.06 (.19)	.11 (.01)	-.07 (.13)	.01 (.88)	.02 (.61)	.04 (.35)	.00 (.99)	.09 (.04)	.04 (.37)	.07 (.11)	.11 (.01)

Upper row = phenotypic correlations
P values for difference from zero in parenthesis

Table 5. Correlations between pH values and Hunter L values of the longissimus dorsi and the semimembranousus.

Trait	24 hr loin pH	48 hr loin pH	Loin Hunter L (24 hr)	Loin Hunter L (48 hr)	Semimembranosus Hunter L (24 hr)
Semimembranosus pH 24 hr	.47 (.0001)	.42 (.0001)	-.002 (.962)	-.16 (.0003)	**-.18** (.0001)
Semimembranosus Hunter L (24 hr)	-.21 (.0001)	-.10 (.0262)	.30 (.0001)	.31 (.0001)	

Upper row = phenotypic correlations
P values for difference from zero in parenthesis

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